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Fatty acid elongases

Abstract:

Nucleic acids are disclosed that encode fatty acid beta-keto acyl synthases from plants. Such synthases are effective for producing very long chain fatty acids (VLCFA), e.g., C22 to C26, preferentially saturated but also monounsaturated. Also disclosed are polypeptides encoded by such nucleic acids. Transgenic plants expressing these polypeptides exhibit altered levels of VLCFA in one or more tissues, such as seeds or leaves.

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(54) Title: FATTY ACID ELONGASES

(57) Abstract

Nucleic acids are disclosed that encode fatty acid β -keto acyl synthases from plants. Such synthases are effective for producing very long chain fatty acids (VLCFA), e.g., C22 to C26, preferentially saturated but also monounsaturated. Also disclosed are polypeptides encoded by such nucleic acids. Transgenic plants expressing these polypeptides exhibit altered levels of VLCFA in one or more tissues, such as seeds or leaves.

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FATTY ACID ELONGASES Field of the Invention

This invention relates to fatty acid elongase complexes and nucleic acids encoding elongase proteins.

More particularly, the invention relates to nucleic acids encoding ß-keto acyl synthase proteins that are effective for producing very long chain fatty acids, polypeptides produced from such nucleic acids and transgenic plants expressing such nucleic acids.

Background of the Invention

Plants are known to synthesize very long chain fatty acids (VLCFAs). VLCFAs are saturated or

15 unsaturated monocarboxylic acids with an unbranched evennumbered carbon chain that is greater than 18 carbons in length. Many VLCFAs are 20-32 carbons in length, but VLCFAs can be up to 60 carbons in length. Important VLCFAs include erucic acid (22:1, i.e., a 22 carbon chain with one double bond), nervonic acid (24:1), behenic acid (22:0), and arachidic acid (20:0).

Plant seeds accumulate mostly 16- and 18-carbon fatty acids. VLCFAs are not desirable in edible oils. Oilseeds of the Crucifereae (e.g., rapeseed) and a few other plants, however, accumulate C20 and C22 fatty acids (FAs). Although plant breeders have developed rapeseed lines that have low levels of VLCFAs for edible oil purposes, even lower levels would be desirable. On the other hand, vegetable oils having elevated levels of VLCFAs are desirable for certain industrial uses, including uses as lubricants, fuels and as a feedstock for plastics, pharmaceuticals and cosmetics.

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The biosynthesis of saturated fatty acids up to an 18-carbon chain occurs in the chloroplast. C2 units from acyl thioesters are linked sequentially, beginning with the condensation of acetyl Coenzyme A (CoA) and malonyl acyl carrier protein (ACP) to form a C4 acyl fatty acid. This condensation reaction is catalyzed by a ß-ketoacyl synthase III (KASIII). ß-ketoacyl moieties are also referred to as 3-ketoacyl moieties.

The enzyme ß-ketoacyl synthase I (KASI) is

10 involved in the addition of C2 groups to form the C6 to
C16 saturated fatty acids. KASI catalyzes the stepwise
condensation of a fatty acyl moiety (C4 to C14) with
malonyl-ACP to produce a 3-ketoacyl-ACP product that is 2
carbons longer than the substrate. The last condensation
15 reaction in the chloroplast, converting C16 to C18, is
catalyzed by ß-ketoacyl synthase II (KASII).

Each elongation cycle involves three additional enzymatic steps in addition to the condensation reaction as discussed above. Briefly, the ß-ketoacyl condensation 20 product is reduced to ß-hydroxyacyl-ACP, dehydrated to the enoyl-ACP, and finally reduced to a fully reduced acyl-ACP. The fully reduced fatty acyl-ACP reaction product then serves as the substrate for the next cycle of elongation.

The C18 saturated fatty acid (stearic acid, 18:0) can be transported out of the chloroplast and converted to the monounsaturate C18:1 (oleic acid), and the polyunsaturates C18:2 (linoleic acid) and C18:3 (α-linolenic acid). C18:0 and C18:1 can also be elongated outside the chloroplast to form VLCFAs. The formation of VLCFAs involves the sequential condensation of two carbon groups from malonyl CoA with a C18:0 or C18:1 fatty acid substrate. Elongation of fatty acids longer than 18 carbons depends on the activity of a fatty acid elongase complex to carry out four separate enzyme reactions

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similar to those described above for fatty acid synthesis in the chloroplast. Fehling, Biochem. Biophys. Acta 1082:239-246 (1991). In plants, elongase complexes are distinct from fatty acid synthases since elongases are extraplastidial and membrane bound.

Mutations have been identified in an Arabidopsis gene associated with fatty acid elongation. This gene, designated the FAE1 gene, is involved in the condensation step of an elongation cycle. See, WO 96/13582,

10 incorporated herein by reference. Plants carrying a mutation in FAE1 have significant decreases in the levels of VLCFAs in seeds. Genes associated with wax biosynthesis in jojoba have also been cloned and sequenced (WO 95/15387, incorporated herein by reference).

Very long chain fatty acids are key components of many biologically important compounds in animals, plants, and microorganisms. For example, in animals, the VLCFA arachidonic acid is a precursor to many prostaglandins.

20 In plants VLCFAs are major constituents of triacylglycerols in many seed oils, are essential precursors for cuticular wax production, and are utilized in the synthesis of glycosylceramides, an important component of the plasma membrane.

Obtaining detailed information on the biochemistry of KAS enzymes has been hampered by the difficulties encountered when purifying membrane bound enzymes.

Although elongase activities have been partially purified from a number of sources, or studied using cell

30 fractions, the elucidation of the biochemistry of elongase complexes has been hampered by the complexity of the membrane fractions used as the enzyme source. For example, until recently, it was unclear as to whether plant elongase complexes were composed of a

35 multifunctional polypeptide similar to the FAS found in

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animals and yeast, or if the complexes existed as discrete and dissociable enzymes similar to the FAS of plants and bacteria. Partial purification of an elongase KAS, immunoblot identification of the hydroxy acyl dehydrase, and the recent cloning of a KAS gene (FAE1) suggest that the enzyme activities of elongase complexes exist on individual enzymes.

Summary of the Invention

The invention disclosed herein relates to an

10 isolated polynucleotide selected from one of the
 following: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID
 NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; an RNA
 analog of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15; and a
 polynucleotide having a nucleic acid sequence
15 complementary to one of the above. The polynucleotide
 can also be a nucleic acid fragment of one of the above
 sequences that is at least 15 nucleotides in length and
 that hybridizes under stringent conditions to genomic DNA
 encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ
20 ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ

Also disclosed herein is an isolated polypeptide that has an amino acid sequence substantially identical to one of the following: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14. Also disclosed are isolated polynucleotides encoding polypeptides substantially identical in their amino acid sequence to: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

ID NO:14.

The invention also relates to a transgenic plant containing a nucleic acid construct. The nucleic acid construct comprises a polynucleotide described above. The construct further comprises a regulatory element

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operably linked to the polynucleotide. The regulatory element may a tissue-specific promoter, for example, an epidermal cell-specific promoter or a seed-specific promoter. The regulatory element may be operably linked to the polynucleotide in sense or antisense orientation. The plant has altered levels of very long chain fatty acids in tissues where the polynucleotide is expressed, compared to a parental plant lacking the nucleic acid construct.

A method is disclosed for altering the levels of 10 very long chain fatty acids in a plant. The method comprises the steps of creating a nucleic acid construct and introducing the construct into the plant. construct includes a polynucleotide selected from one of 15 the following: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; an RNA analog of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15; and a polynucleotide having a nucleic acid sequence complementary to one of the above. The polynucleotide 20 can also be a nucleic acid fragment of one of the above that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ 25 ID NO:14. The polynucleotide is effective for altering the levels of very long chain fatty acids in the plant.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

30

Brief Description of the Drawings

Figure 1 shows the time course of $in\ vitro\ \text{VLCFA}$ synthesis by FAE1 expressed in yeast, with 3 different acyl-CoA substrates.

Figure 2 shows the rates of *in vitro* VLCFA synthesis and the VLCFA profiles of *FAE1* expressed in yeast, with 3 different acyl-CoA substrates.

Figure 3 shows the nucleotide sequence of the 5 coding region of the *Arabidopsis* EL1 polynucleotide (SEQ ID NO:1).

Figure 4 shows the deduced amino acid sequence (SEQ ID NO:2) for the EL1 coding sequence of Figure 3.

Figure 5 shows the nucleotide sequence of the 10 coding region of the Arabidopsis EL2 polynucleotide (SEQ ID NO:3).

Figure 6 shows the deduced amino acid sequence (SEQ ID NO:4) for the EL2 coding sequence of Figure 5.

Figure 7 shows the nucleotide sequence of the 15 coding region of the Arabidopsis EL3 polynucleotide (SEQ ID NO:5).

Figure 8 shows the deduced amino acid sequence (SEQ ID NO:6) for the EL3 coding sequence of Figure 7.

Figure 9 shows the nucleotide sequence of the 20 coding region of the Arabidopsis EL4 polynucleotide (SEQ ID NO:7).

Figure 10 shows the deduced amino acid sequence (SEQ ID NO:8) for the EL4 coding sequence of Figure 9.

Figure 11 shows the nucleotide sequence of the 25 coding region of the Arabidopsis EL5 polynucleotide (SEQ ID NO:9).

Figure 12 shows the deduced amino acid sequence (SEQ ID NO:10) for the EL5 coding sequence of Figure 11.

Figure 13 shows the nucleotide sequence of the 30 coding region of the Arabidopsis EL6 polynucleotide (SEQ ID NO:11).

Figure 14 shows the deduced amino acid sequence (SEQ ID NO:12) for the EL6 coding sequence of Figure 13.

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Figure 15 shows the nucleotide sequence of the coding region of the Arabidopsis EL7 polynucleotide (SEQ ID NO:13).

Figure 16 shows the deduced amino acid sequence 5 (SEQ ID NO:14) for the EL7 coding sequence of Figure 15.

Description of the Preferred Embodiments

The present invention comprises isolated nucleic acids (polynucleotides) that encode polypeptides having ß-ketoacyl synthase activity. The novel polynucleotides and polypeptides of the invention are involved in the synthesis of very long chain fatty acids and are useful for modulating the total amounts of such fatty acids and the specific VLCFA profile in plants.

A polynucleotide of the invention may be in the

15 form of RNA or in the form of DNA, including cDNA,
synthetic DNA or genomic DNA. The DNA may be doublestranded or single-stranded, and if single-stranded, can
be either the coding strand or non-coding strand. An RNA
analog may be, for example, mRNA or a combination of

20 ribo- and deoxyribonucleotides. Illustrative examples of
a polynucleotide of the invention are shown in Figs. 3,
5, 7, 9, 11, 13 and 15.

A polynucleotide of the invention typically is at least 15 nucleotides (or base pairs, bp) in length. In 25 some embodiments, a polynucleotide is about 20 to 100 nucleotides in length, or about 100 to 500 nucleotides in length. In other embodiments, a polynucleotide is greater than about 1500 nucleotides in length and encodes a polypeptide having the amino acid sequence shown in 30 Figs. 4, 6, 8, 10, 12, 14 or 16.

In some embodiments, a polynucleotide of the invention encodes analogs or derivatives of a polypeptide having the deduced amino acid sequence of Figs. 4, 6, 8,

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10, 12, 14 or 16. Such fragments, analogs on derivatives include, for example, naturally occurring allelic variants, non-naturally occurring allelic variants, deletion variants and insertion variants, that do not substantially alter the function of the polypeptide.

A polynucleotide of the invention may further comprise additional nucleic acids. For example, a nucleic acid fragment encoding a secretory or leading amino acid sequence can be fused in-frame to the amino terminal end of one of the EL1 through EL7 polypeptides. Other nucleic acid fragments are known in the art that encode amino acid sequences useful for fusing in-frame to the KAS polypeptides disclosed herein. See, e.g., U.S. 5,629,193 incorporated herein by reference. A polynucleotide may further comprise one or more regulatory elements operably linked to a KAS polynucleotide disclosed herein.

The present invention also comprises polynucleotides that hybridize to a KAS polynucleotide 20 disclosed herein. Such a polynucleotide typically is at least 15 nucleotides in length. Hybridization typically involves Southern analysis (Southern blotting), a method by which the presence of DNA sequences in a target nucleic acid mixture are identified by hybridization to a 25 labeled oligonucleotide or DNA fragment probe. Southern analysis typically involves electrophoretic separation of DNA digests on agarose gels, denaturation of the DNA after electrophoretic separation, and transfer of the DNA to nitrocellulose, nylon, or another suitable membrane 30 support for analysis with a radiolabeled, biotinylated, or enzyme-labeled probe as described in sections 9.37-9.52 of Sambrook et al., (1989) Molecular Cloning, second edition, Cold Spring Harbor Laboratory, Plainview; NY.

A polynucleotide can hybridize under moderate 35 stringency conditions or, preferably, under high

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stringency conditions to a KAS polynucleotide disclosed High stringency conditions are used to identify nucleic acids that have a high degree of homology to the probe. High stringency conditions can include the use of 5 low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate (0.1X SSC); 0.1% sodium lauryl sulfate (SDS) at 65°C. Alternatively, a denaturing agent such as formamide can be employed during hybridization, e.g., 50% formamide with 0.1% 10 bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is the use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium 15 phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 $\mu q/ml$), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Moderate stringency conditions refers to
20 hybridization conditions used to identify nucleic acids
that have a lower degree of identity to the probe than do
nucleic acids identified under high stringency
conditions. Moderate stringency conditions can include
the use of higher ionic strength and/or lower
25 temperatures for washing of the hybridization membrane,
compared to the ionic strength and temperatures used for
high stringency hybridization. For example, a wash
solution comprising 0.060 M NaCl/0.0060 M sodium citrate
(4X SSC) and 0.1% sodium lauryl sulfate (SDS) can be used
30 at 50°C, with a last wash in 1X SSC, at 65°C.
Alternatively, a hybridization wash in 1X SSC at 37°C can
be used.

Hybridization can also be done by Northern analysis (Northern blotting), a method used to identify 35 RNAs that hybridize to a known probe such as an

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oligonucleotide, DNA fragment, cDNA or fragment thereof, or RNA fragment. The probe is labeled with a radioisotope such as ³²P, by biotinylation or with an enzyme. The RNA to be analyzed can be usually electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe, using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook 10 et al., supra.

A polynucleotide has at least about 70% sequence identity, preferably at least about 80% sequence identity, more preferably at least about 90% sequence identity to SEQ ID NO:1, 3, 5, 7, 9, 11, or 13. Sequence identity can be determined, for example, by computer programs designed to perform single and multiple sequence alignments.

A polynucleotide of the invention can be obtained by chemical synthesis, isolation and cloning from plant 20 genomic DNA or other means known to the art, including the use of PCR technology carried out using oligonucleotides corresponding to portions of SEQ ID NO:1, 3, 5, 7-9, 11 or 13. Polymerase chain reaction (PCR) refers to a procedure or technique in which target 25 nucleic acid is amplified in a manner similar to that described in U.S. Patent No. 4,683,195, incorporated herein by reference, and subsequent modifications of the procedure described therein. Generally, sequence information from the ends of the region of interest or 30 beyond is employed to design oligonucleotide primers that are identical or similar in sequence to opposite strands of the template to be amplified. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total 35 cellular RNA, bacteriophage or plasmid sequences, and the

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like. Alternately, a cDNA library (in an expression vector) can be screened with KAS-specific antibody prepared using peptide sequence(s) from hydrophilic regions of the KAS protein of SEQ ID NO:2 and technology 5 known in the art.

A polypeptide of the invention comprises an isolated polypeptide having the deduced amino acid sequence of Figs. 2, 4, 6, 8, 10 and 12, as well as derivatives and analogs thereof. By "isolated" is meant a polypeptide that is expressed and produced in an environment other than the environment in which the polypeptide is naturally expressed and produced. For example, a plant polypeptide is isolated when expressed and produced in bacteria or fungi. Similarly, a plant polypeptide is isolated when its gene coding sequence is operably linked to a chimeric regulatory element and expressed in a tissue where the polypeptide is not naturally expressed. A polypeptide of the invention also comprises variants of the KAS polypeptides disclosed herein, as discussed above.

A full-length KAS coding sequence may comprise the sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13.

Alternatively, a chimeric full-length KAS coding sequence may be formed by linking, in-frame, nucleotides from the 25 5' region of a first KAS gene to nucleotides from the 3' region of a second KAS gene, thereby forming a chimeric KAS protein.

It should be appreciated that nucleic acid fragments having a nucleotide sequence other than the KAS sequences disclosed in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 will encode a polypeptide having the exemplified amino acid coding sequence of SEQ ID NO:2, 4, 6, 8, 10, 12 or 14, respectively. The degeneracy of the genetic code is well-known to the art; i.e., for many amino acids, there

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is more than one nucleotide triplet which serves as the codon for the amino acid.

It should also be appreciated that certain amino acid substitutions can be made in protein sequences 5 without affecting the function of the protein. Generally, conservative amino acid substitutions or substitutions of similar amino acids are tolerated without affecting protein function. Similar amino acids can be those that are similar in size and/or charge 10 properties, for example, aspartate and glutamate and isoleucine and valine are both pairs of similar amino acids. Similarity between amino acid pairs has been assessed in the art in a number of ways. For example, Dayhoff et al. (1978) in Atlas of Protein Sequence and 15 Structure, Vol. 5, Suppl. 3, pp. 345-352, which is incorporated by reference herein, provides frequency tables for amino acid substitutions which can be employed as a measure of amino acid similarity.

A nucleic acid construct of the invention

20 comprises a polynucleotide as disclosed herein linked to
another, different polynucleotide. For example, a fulllength KAS coding sequence may be operably fused in-frame
to a nucleic acid fragment that encodes a leader
sequence, secretory sequence or other additional amino

25 acid sequences that amy be usefully linked to a
polypeptide or peptide fragment.

A transgenic plant of the invention contains a nucleic acid construct as described herein. In some embodiments, a transgenic plant contains a nucleic acid construct that comprises a polynucleotide of the invention operably linked to at least one suitable regulatory sequence in sense orientation. Regulatory sequences typically do not themselves code for a gene product. Instead, regulatory sequences affect the expression level of the polynucleotide to which they are

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linked. Examples of regulatory sequences are known in the art and include, without limitation, minimal promoters and promoters of genes preferentially or exclusively expressed in seeds or in epidermal cells of stems and leaves. Native regulatory sequences of the polynucleotides disclosed herein can be readily isolated by those skilled in the art and used in constructs of the invention. Other examples of suitable regulatory sequences include enhancers or enhancer-like elements, introns, 3' non-coding regions such as poly A sequences and other regulatory sequences discussed herein.

Molecular biology techniques for preparing such chimeric genes are known in the art.

In other embodiments, a transgenic plant contains
15 a nucleic acid construct comprising a partial or a fulllength KAS coding sequence operably linked to at least
one suitable regulatory sequence in antisense
orientation. The chimeric gene can be introduced into a
plant and transgenic progeny displaying expression of the
20 antisense construct are identified.

One may use a polynucleotide disclosed herein for cosuppression as well as for antisense inhibition.

Cosuppression of genes in plants may be achieved by expressing, in the sense orientation, the entire or partial coding sequence of a gene. See, e.g., WO 04\11516, incorporated herein by reference.

Transgenic techniques for use in the invention include, without limitation, Agrobacterium-mediated transformation, viral vector-mediated transformation electroporation and particle gun transformation.

Illustrative examples of transformation techniques are described in U.S. Patent 5,204,253, (particle gun) and U.S. Patent 5,188,958 (Agrobacterium), incorporated herein by reference. Transformation methods utilizing the Ti and Ri plasmids of Agrobacterium spp. typically

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use binary-type vectors. Walkerpeach, C. et al., in Plant Molecular Biology Manual, S. Gelvin and R. Schilperoort, eds., Kluwer Dordrecht, C1:1-19 (1994). If cell or tissue cultures are used as the recipient tissue for transformation, plants can be regenerated from transformed cultures by techniques known to those skilled in the art.

Techniques are known for the introduction of DNA into monocots as well as dicots, as are the techniques

10 for culturing such plant tissues and regenerating those tissues. Monocots which have been successfully transformed and regenerated include wheat, corn, rye, rice, and asparagus. See, e.g., U.S. Patent Nos.

5,484,956 and 5,550,318, incorporated herein by reference.

For efficient production of transgenic plants from plant cells, it is desirable that the plant tissue used for transformation possess a high capacity for regeneration. Transgenic plants of woody species such as 20 poplar and aspen have also been obtained. Technology is also available for the manipulation, transformation, and regeneration of gymnosperm plants. For example, U.S. Patent No. 5,122,466 describes the biolistic transformation of conifers, with preferred target tissue being meristematic and cotyledon and hypocotyl tissues. U.S. Patent No. 5,041,382 describes enrichment of conifer embryonal cells.

Seeds produced by a transgenic plant(s) can be grown and then selfed (or outcrossed and selfed) to obtain seeds homozygous for the construct. Seeds can be analyzed in order to identify those homozygotes having the desired expression of the construct. Transgenic plants may be entered into a breeding program, e.g., to introgress the novel construct into other lines, to transfer the construct to other species, or for further

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selection of other desirable traits. Alternatively, transgenic plants may be propagated vegetatively for those species amenable to such techniques. A nucleic acid construct of the invention can alter the levels of 5 very long chain fatty acids in plant tissues expressing the polynucleotide, compared to VLCFA levels in corresponding tissues from an otherwise identical plant not expressing the polynucleotide. A comparison can be made, for example, between a non-transgenic plant of a 10 plant line and a transgenic plant of the same plant line. Levels of VLCFAs having 20-32 carbons and/or levels of VLCFAs having 32-60 carbons can be altered in plants disclosed herein. Plants having an altered VLCFA composition may be identified by techniques known to the 15 skilled artisan, e.g., thin layer chromatography or gasliquid chromatography (GLC) analysis of the appropriate plant tissue.

A suitable group of plants with which to practice the invention are the *Brassica* species, including *B*.

20 napus, *B.* rapa, *B.* juncea, and *B.* hirta. Other suitable plants include, without limitation, soybean (*Glycine max*), sunflower (*Helianthus annuus*) and corn (*Zea mays*).

A method according to the invention comprises introducing a nucleic acid construct into a plant cell and producing a plant (as well as progeny of such a plant) from the transformed cell. Progeny includes descendants of a particular plant or plant line, e.g., seeds developed on an instant plant are descendants. Progeny of an instant plant include seeds formed on F₁, 30 F₂, F₃, and subsequent generation plants, or seeds formed on BC₁, BC₂, BC₃, and subsequent generation plants.

Methods and compositions according to the invention are useful in that the resulting plants and plant lines have desirable alterations in very long chain 35 fatty acid composition. Suitable tissues in which to

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express polynucleotides and/or polypeptides of the invention include, without limitation, seeds, stems and leaves. Leaf tissues of interest include cells and tissues of the epidermis, e.g., cells that are involved in forming trichomes. Of particular interest are epidermal cells involved in forming the cuticular layer. The cuticular layer comprises various very long chain fatty acids and VLCFA derivatives such as alkanes, esters, alcohols and aldehydes. Altering the composition and amount of VLCFAs in epidermal cells and tissues may enhance defense mechanisms and drought tolerance of plants disclosed herein.

Polynucleotides of the invention can be used as markers in plant genetic mapping and plant breeding
15 programs. Such markers may include RFLP, RAPD, or PCR markers, for example. Marker-assisted breeding techniques may be used to identify and follow a desired fatty acid composition during the breeding process.

Marker-assisted breeding techniques may be used in
20 addition to, or as an alternative to, other sorts of identification techniques. An example of marker-assisted breeding is the use of PCR primers that specifically amplify a sequence from a desired KAS that has been introduced into a plant line and is being crossed into other plant lines.

Plants and plant lines disclosed herein preferably have superior agronomic properties. Superior agronomic characteristics include, for example, increased seed germination percentage, increased seedling vigor,

30 increased resistance to seedling fungal diseases (damping off, root rot and the like), increased yield, and improved standability.

While the invention is susceptible to various modifications and alternative forms, certain specific embodiments thereof are described in the general methods

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and examples set forth below. It should be understood, however, that these examples are not intended to limit the invention to the particular forms disclosed but, instead the invention is to cover all modifications, equivalents and alternatives falling within the scope of the invention.

EXAMPLES

Example 1

Cloning and Expression of FAE1 in Yeast Cells

The open reading frame of the Arabidopsis FAE1 gene was amplified directly by PCR, using Arabidopsis thaliana cv. Columbia genomic DNA as a template, pfu DNA polymerase and the following primers:

5'CTCGAGGAGCAATGACGTCCGTTAA-3' and 5'-

15 CTCGAGTTAGGACCGACCGTTTTG-3'. The PCR product was blunt-end cloned into the *Eco* RV site of pBluescript (Stratagene, La Jolla, CA),

The FAE1 gene was excised from the Bluescript vector with BamH1, and then subcloned into the pYEUra3

20 (Clontech, Palo Alto, CA). pYEUra3 is a yeast centromere-containing, episomal plasmid that is propagated stably through cell division. The FAE1 gene was inserted downstream of a GAL1 promoter in pYEUra3. The GAL1 promoter is induced when galactose is present in the growth medium.

Insertion of the FAE1 gene in the sense orientation was confirmed by PCR, and pYEUra3/FAE1 was used to transform Saccharomyces cerevisiae strain AB1380 using a lithium acetate procedure as described in Gietz, R. and Woods, R., in Molecular Genetics of Yeast: Practical Approaches, Oxford Press, pp. 121-134 (1994). Plasmid DNA was isolated from putative transformants, and

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the presence of the FAE1/pYEUra3 construct was confirmed by Southern analysis.

Yeast transformed with pYEUra3 having FAE1 operably linked to the GAL1 promoter were grown in the 5 presence of galactose or glucose and were analyzed for the expression of FAE1. As a control, yeast transformed with pYEUra3 containing no insert were also assayed. Analysis of such control preparations yielded fatty acid compositions and fatty acid elongation rates similar to those of yeast transformed with pYEUra3/FAE1 and grown with glucose as the carbon source.

The fatty acid composition of yeast cells grown in the presence of galactose was compared to that of cells grown in the presence of glucose, to determine if VLCFA were found in the galactose-induced cells.

Transformed yeast cells were grown overnight in YPD media at 30°C with vigorous shaking. One hundred μl of the overnight culture were used to inoculate 40 ml of complete minimal uracil dropout media (CM-Ura)

- supplemented with either glucose or galactose (2% $\rm w/v$). Cultures were grown at 30°C to an OD₆₀₀ of approximately 1.3 to 1.5. Cells were harvested by centrifugation at 5000 Xg for 10 min. Total lipids were extracted from the cells with 2 volumes of 4N KOH in 100% methanol for 60
- 25 min. at 80°C. Fatty acids were saponified and methyl esters were prepared by drying the samples and resuspending in 0.5 ml of boron trichloride in methanol (10% v/v). Samples were incubated at 50°C for 15 min in a sealed tube. About 2 ml of water was then added and
- of hexane. Extracts were extracted thrice with 1 ml of hexane. Extracts were dried under nitrogen and redissolved in hexane. See Hlousek-Radojcic, A. et al., Plant J. 8:803-809. Methyl esters were analyzed on an HP 5890 series II gas chromatograph equipped with a 5771MSD and 7673 auto injector (Hewlett-Packard, Cincinnati, OH).

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Methyl esters were separated on a DB-23 (J&W Scientific) capillary column (30 m X 0.25 mm X 0.25 μm). The column was operated with helium carrier gas and splitless injection (injection temperature 280°C, detector temperature 280°C). After an initial 3 min. at 100°C, the oven temperature was raised to 250° at 20°C min⁻¹ and maintained at that temperature for an additional 3 min. The identity of the peaks was verified by cochromatography with authentic standards and by mass spectrometer analysis.

The results clearly revealed the appearance of both 20:1 and 22:1 acyl-CoA products in galactose-induced yeast containing the FAE1 coding sequence. Uninduced yeast cells failed to accumulated significant amounts of fatty acids longer than C18. These results indicate that expression of FAE1 in yeast resulted in functional KAS activity and functional elongase activity.

Example 2

FAE1 Activity in Yeast Microsomes

The functional expression of the FAE1 KAS was analyzed by isolating microsomes from transformed yeast cells and assaying these microsomes in vitro for elongase activity.

Transformed yeast cells were grown in the presence of either glucose or galactose (2% w/v) as described in Example 1. Cells were harvested by centrifugation at 5000 Xg for 10 min and washed with 10 ml ice cold isolation buffer (IB), which contains 80 mM Hepes-KOH, pH 7.2, 5 mM EGTA, 5 mM EDTA, 10 mM KCl, 320 mM sucrose and 2 mM DTT). Cells were then resuspended in enough IB to fill a 1.7 ml tube containing 700 μ l of 0.5 μ m glass beads and yeast microsomes were isolated from the cells essentially as described in Tillman, T. and Bell, R., J. Biol. Chem. 261:9144-9149 (1986). The microsomal

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membrane pellet was recovered by centrifugation at 252,000 xg for 60 min. The pellet was rinsed by resuspending in 40 ml fresh IB and again recovered by centrifugation at 252000 Xg for 60 min. Microsomal pellets were resuspended in a minimal volume of IB, and the protein concentration adjusted to 2.5 μg μl⁻¹ by addition of IB containing 15% glycerol. Microsomes were frozen on dry ice and stored at -80°C. The protein concentration in microsomes was determined by the Bradford method (Bradford, 1976).

Fatty acid elongase activity was measured essentially as described in Hlousek-Radojcic, A. et al., Plant J. 8:803-809 (1995). Briefly, the standard elongation reaction mix contained 80 mM Hepes-KOH, pH 7.2, 20 mM MgCl₂, 500 μ M NADPH, 1 mM ATP, 100 uM malonyl-CoA, 10 μ M CoA-SH and 15 μ M radioactive acyl-CoA substrate. The radiolabeled substrate was either [1 14 C]18:1-CoA (50 uCi μ mol⁻¹), [1- 14 C]18:0-CoA (55 uCi μ mol⁻¹), or [1- 14 C]16:0-CoA (54 uCi μ mol⁻¹). The reaction was initiated by the addition of yeast microsomes (5 μ g protein) and the mixture incubated at 30° C for the indicated period of time. The final reaction volume was 25 μ l.

Methyl esters of the acyl-CoA elongation products

were prepared as described in Example 1. Methyl esters
were separated on reversed phase silica gel KC18 TLC

plates (Whatman, 250 uM thick), quantified by
phosphorimaging, and analyzed on by ImageQuant software
(Molecular Dynamics, Inc., Sunnyvale, CA). The detection

limit for each product is about 0.001 nanomoles per min.
per mg microsomal protein, depending on the phosphorimage
exposure time.

Results of representative in vitro elongation assays are shown in Figs. 1 and 2. The results indicate that microsomes from galactose-induced cells expressing

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FAE1 catalyzed multiple cycles of elongation starting with either C16:0 acyl CoA, C18:0 acyl CoA, or C18:1 acyl-CoA as the substrate (Fig. 1). The 16:0 and 18:0 acyl-CoA substrates were elongated to C26:0 acyl-CoA. In contrast, the 18:1-CoA substrate was elongated primarily to C20:1, with only low levels of C22:1 acyl-CoA being produced. Occasionally, trace levels of C24:1 CoA were also observed. Although the chain length of the products from the 18:1 acyl-CoA substrate were less than the chain length from the saturated acyl-CoA substrates, the rate of elongation of oleoyl-CoA was about 2- and 3-fold higher than the rates of elongation of 16:0-CoA and 18:0-CoA, respectively.

The elongation activity observed in microsomes

from uninduced cells indicated that there was a low level of endogenous elongase activity when 18:1-CoA or 18:0-CoA were used as substrates. There was substantial 16:0-CoA elongase activity (10.1 nmol mg protein-1 at 30 min) in microsomes from uninduced cells (Fig. 2). However, the

major product of 16:0 elongation using uninduced microsomes was C18:0 acyl CoA, with only small amounts of products beyond this length. The elongation of the 16:0 acyl-CoA substrate presumably is due to an endogenous yeast elongase.

Elongation of 18:1 CoA by microsomes from induced cells occurred at a rate about 18-fold higher than in microsomes isolated from the uninduced cells (Fig. 2). With microsomes from induced yeast, synthesis of 20:0 CoA from the 16:0 CoA substrate, occurred at a rate similar to that seen when the substrate was 18:0 CoA (4.2 vs. 5.1 nmol mg protein⁻¹). The total rate of elongation of [14C] 16:0-CoA by microsomes from induced cells (15.8 nmol mg protein⁻¹ at 30 min.) was more than 50% higher than elongation of [14C] 16:0-CoA by microsomes from uninduced cells, suggesting that the FAE1 KAS utilized 16:0-CoA as

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a substrate in addition to C18-C24 acyl-CoAs. The FAE1 elongase KAS activity, i.e., the difference in the 16:0 elongation rates between microsomes from induced and uninduced cells, was 5.7 nmol mg protein⁻¹. The elongation rate with the 16:0 substrate thus was similar to the elongase activity of the FAE1 elongase KAS with the 18:0 substrate.

These results indicate that FAE1 KAS expressed in yeast could synthesize 3-ketoacyl-CoA in vitro and, in combination with yeast reductases and dehydrases, could form a functional VLCFA elongase complex. In addition, these results suggest that FAE1 is membrane-bound in yeast cells.

Example 3

15 Cloning and Sequencing of Arabidopsis Elongase Genes

The sequence of a jojoba seed cDNA (see WO 93/10241 and WO 95/15387, incorporated herein by reference) was used to search the Arabidopsis expressed sequence tag (EST) database of the Arabidopsis Genome 20 Stock Center (The Ohio State University, Columbus, Ohio). The BLAST computer program (National Institutes of Health, Bethesda, MD, USA) was used to perform the search. The search identified two ESTs (ATTS1282 and ATTS3218) that had a high degree of sequence identity with the jojoba sequence. The ATTS1282 and ATTS3218 ESTs appeared to be partial cDNA clones rather than full-length clones based on the length of the jojoba sequence.

A genomic DNA library from Arabidopsis thaliana cv. Columbia, was prepared in the lambda GEM11 vector 30 (Promega, Madison, Wisconsin) and was obtained from Ron Davis, Stanford University, Stanford, CA. The library was hybridized with ATTS1282 and ATTS3218 as probes and 2 clones were identified for each EST. Phage DNA was isolated from each of the hybridizing clones, the genomic

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insert was excised with the restriction enzyme Sac I and subcloned into the plasmid pBluescript (Stratagene, La Jolla, CA). One clone from the ATTS1282 hybridization was designated EL1 and one clone from the ATTS3218

5 hybridization was designated EL2.

A yeast expression library, containing cDNA from Arabidopsis thaliana cv. Columbia, was prepared in the lambda YES expression vector described in Elledge et al. (Elledge, S. et al., Proc. Natl. Acad. Sci USA 88:1731-10 1735 (1991) and was obtained from Ron Davis at Stanford University, Stanford, CA. The library was hybridized with a EL2 partial cDNA probe. A full-length EL2 cDNA was not identified. However, the probe did identify a full-length cDNA which was designated EL3.

A consensus sequence for the C-terminal region of EL1, EL2 and the jojoba cDNA polypeptides was identified by sequence alignment using DNA analysis programs from DNAStar, Madison, Wisconsin. This consensus sequence was used to search the Arabidopsis EST database again for ß-20 keto acyl synthase sequences. These searches identified four additional putative ß-keto acyl synthase ESTs, which were designated EL4 through EL7. EL4, EL5, EL6, and EL7 have homology to Genbank Accession Nos. T04345, T44939, T22193 and T76700, respectively.

The lambda YES cDNA expression library described above was hybridized with the EL1 and EL4-EL7 ESTs as probes. This screen identified full-length cDNAs for EL1, EL5 and EL6.

The lambda GEM11 genomic library was hybridized 30 with the EL4 and EL7 ESTs as probes. This screen identified full-length genomic clones for EL4 and EL7. Phage DNA was isolated from each of the hybridizing clones and subcloned into pBluescript as described above.

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The 7 EL clones were sequenced on both strands with regions of overlap for each sequence run.

Sequencing was carried out with an ABI automated sequencer (Applied Biosystems, Inc., Foster City,

5 California), following the manufacturer's instructions.

The nucleotide sequences for the coding regions of EL1-EL7 are shown in Figs. 3, 5, 7, 9, 11, 13 and 15, respectively. The deduced amino acid sequences for EL1-EL7 are shown in Figs. 4, 6, 8, 10, 12, 14 and 16,

10 respectively, using the standard one-letter amino acid code. The EL1, EL2 and EL7 genomic clones appeared to lack introns. The EL4 genomic clone contained one intron near the 5' end of the coding region.

The nucleotide sequences of the 7 EL

15 polynucleotides were compared to 5 DNA sequences present in Genbank. Genbank, National Center for Biotechnology Information, Bethesda, MD. Two of the 5 accessions were cloned from members of the Brassicaceae: the Arabidopsis FAE1 sequence (Accession U29142) and a Brassica napus 20 sequence (Accession U50771). Three of the accessions were cloned from jojoba (Simmondsia chinensis): 2 wax biosynthesis genes (Accessions I14084 and I14085) and a jojoba KAS gene (Accession U37088). See also U.S. Patent 5,445,947, incorporated herein by reference.

Multiple alignment of the 12 sequences was carried out with a computer program sold under the trade name MEGALIGN Lasergene by DNAStar (Madison, Wisconsin). Alignments were done using the Clustal method with weighted residue weight table. The nucleotide sequence similarity index and percent divergence based on the multiple alignment algorithm is shown in Table 1. The nucleotide sequences of EL1-EL7 are distinguishable from the 5 DNA sequences obtained from Genbank.

The deduced amino acid sequences of the EL1-7 35 polypeptides were compared with the MEGALIGN program to

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the deduced amino acid sequences of the same 5 Genbank clones, using the Clustal method with PAM250 residue weight table. The amino acid sequence similarity and percent divergence are shown in Table 2. The amino acid sequences of EL1-EL7 polypeptides are distinguishable from those of the Genbank sequences.

TABLE 1

Nucleotide sequence pair distances of EL1-EL7, using Clustal method with weighted residue weight table.

	29142	50771						37088	14084	14085			
	ARAFAE1 U29142	BNaFAE1 U50771	61	_				JOJOKCS U37088	JOKCS10 I14084	JOKCS11 I14085			
	ARZ	BN	EL2	EL3	ELS	EL7	EL6	Š	JOK	JOK	ELI	EL4	
	н	73	т	4	S	9	7	8	D	10	11	12	
12	41.3	40.5	43.5	42.3	47.2	49.2	48.2	45.8	44.8	45.3	48.3		12
11	44.7	42.3	46.5	47.4	49.0	49.0	47.7	48.4	47.6	48.4		59.9	11
10	43.1	42.9	48.6	47.2	46.4	48.6	49.8	99.7	95.9		69.9	73.3	10
6	42.9	44.1	48.1	45.1	46.6	48.2	49.2	97.7		1.1	71.1	73.8	δ
8	42.8	42.7	48.5	47.0	46.8	48.6	49.8		1.1	0.2	71.1	73.4	œ
7	47.0	46.9	46.7	46.5	54.0	53.6		56.1	56.6	56.3	83.1	91.9	7
9	54.9	53.7	56.4	55.4	68.0		64.5	64.2	64.1	63.0	82.4	82.8	9
5	57.0	55.4	59.3	56.3		32.4	64.3	65.5	64.6	64.1	77.4	84.5	5
4	58.8	57.9	70.5		45.0	47.3	67.3	63.1	64.6	61.4	77.0	91.5	4
3	62.4	61.0		28.0	45.0	46.0	69.4	63.4	63.7	61.8	81.0	95.4	3
2	77.5		41.0	44.3	42.3	48.9	71.0	66.2	65.4	65.2	85.8	90.4	2
1		18.1	40.4	43.9	40.7	45.8	74.1	68.1	67.0	67.2	88.6	95.7	H
	1	2	3	4	5	9	7	80	9	10	11	12	

TABLE 2

Amino acid sequence pair distances of EL1-EL7, using Clustal method with PAM250 residue weight table.

	EL2	EL3	ATFAE1 U29142	BNFAE1 U50771	EL7	ELS	EL6	JOJKCS U37088	JKCS11 I14085	JKCS10 I14084	EL1	EL4	
	1	77	ъ	4	5	9	7	8	o	10	11	12	
12	42.0	44.4	43.9	42.4	55.6	50.5	51.6	52.0	51.9	50.7	50.8		12
11	49.1	49.6	47.8	46.5	55.0	52.9	53.4	53.1	53.1	51.7		69.4	11
10	51.5	49.2	50.8	49.7	58.3	54.9	51.8	96.9	96.9		65.3	69.9	10
6	52.1	50.0	51.6	50.5	58.9	55.7	52.8	99.8		1.6	63.9	68.5	Q
8	51.9	49.8	51.4	50.3	58.7	55.7	52.8		0.2	1.8	63.9	68.5	8
7	50.3	49.8	50.0	49.2	61.0	61.8		67.7	67.7	68.6	67.2	67.1	7
9	60.2	57.1	63.0	61.0	75.8		50.8	59.8	59.3	60.7	66.0	70.8	9
5	60.9	58.7	60.7	60.2		29.3	52.0	54.8	54.0	54.5	60.8	9.09	5
4	59.8	57.5	82.4		46.2	46.5	74.4	67.3	67.3	67.8	74.4	83.3	4
3	62.9	60.1		17.9	45.8	42.8	71.8	66.2	66.2	9.99	72.8	82.7	3
2	72.0		48.7	52.8	51.3	55.5	70.5	69.2	68.7	69.7	73.7	85.5	2
1		31.1	47.4	51.8	49.0	52.6	74.7	66.7	66.3	66.3	73.6	84.8	1
	1	2	3	4	2	9	7	8	6	10	11	12	

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Example 4

Expression of EL1 and EL2 in Yeast

The open reading frames (ORFs) for the EL2, EL4 and EL7 clones were amplified by PCR. The EL2 ORF was cloned into \(\lambda\)YES using the primers:
CTCGAGCAAGTCCACTACCACGCA and CTCGAGCGAGTCAGAAGGAACAAA.
The EL4 ORF was cloned into pYEUra3 using the primers:
GATAATTTAGAGAGGCACAGGGT and GTCGACACAAGAATGGGTAGATCCAA.
The EL7 ORF was cloned into pYEUra3 using the primers:
CAGTTCCTCAAACGAAGCTA and GTCGACTTCTCAATGGACGGTGCCGGA.
Amplified products were cloned into pYEUra3 under the control of, and 3' to, the GAL1 promoter. The resulting plasmids were transformed into yeast as described in Example 1.

Yeast cultures containing full-length EL1 in λ YES and full-length EL2 in pYEUra3 were grown in the presence of galactose or glucose as described in Example 2. Microsomes were then prepared from each of the cultures and fatty acid elongation assays were carried out as described in Example 2.

In the first experiment, microsomes were prepared from galactose-induced cultures of EL1, EL2 and FAE1, and incubated with either [1-14C] 18:0 acyl-CoA or [1-14C] 18:1 acyl-CoA as substrate. The amounts of various reaction products synthesized after 30 minutes (min) were determined as described in Example 2. The results when 18:0 acyl-CoA was the substrate are shown in Table 3. The results when 18:1 acyl-CoA was the substrate are shown in Table 4.

Table 3.
Elongation of 18:0-CoA by FAE1, EL1 and EL2 Genes
Expressed in Yeast

			ß-Keto Acyl Sy	nthase Gene			
	FA	E1	EL	1	EL2		
Acyl-CoA Product	Rate ¹	(%)	Rate	(%)	Rate	(%)	
20:0	0.369	64.3	0.084	38.8	0.108	41.8	
22:0	0.113	18.6	0.047	21.9	0.053	20.7	
24:0	0.065	10.7	0.043	19.9	0.052	20.3	
26:0	0.038	6.3	0.042	19.4	0.044	17.2	
Total	0.585	100.0	0.216	100.0	0.258	100.0	

¹ Nanomoles/minute/mg of microsomal protein

Table 4.
Elongation of 18:1-CoA by FAE1, EL1 and EL2 Genes
Expressed in Yeast

	ß-Keto Acyl Synthase Gene											
	FAI	E1	EL	1	EL2							
Acyl-CoA Product	Rate¹	(%)	Rate	(%)	Rate	(%)						
20:1	1.131	84.6	0.111	80.8	0.091	84.1						
22:1	0.206	15.4	0.026	19.2	0.017	15.9						
24:1	0.0	0.0	0.0	0.0	0.0	0.0						
26:1	0.0	0.0	0.0	0.0	0.0	0.0						
Total	1.337	100.0	0.137	100.0	0.108	100.0						

¹ Nanomoles/minute/mg of microsomal protein

The results shown in Tables 3 and 4 indicate that the EL1 and EL2 gene products have ß-ketoacyl synthase (KAS) activity and that the KAS reaction product can be utilized to form VLCFAs. The specific activities of the 3 KAS enzymes cannot be compared, since the relative amount of the heterologous KAS protein in each microsomal preparation is not known. However, the proportions of various reaction products can be compared between FAE1, EL1 and EL2.

The data shown in Table 3 indicate that the EL1 and EL2 KAS activities result in a higher proportion of saturated VLCFAs than does the FAE1 KAS activity. These

results suggest that EL1 and EL2 encode novel gene products, because EL1 and EL2 have a greater preference for C22:0 and C24:0 acyl-CoA substrates than does FAE1.

A comparison of the relative elongation activity of FAE1 with 18:0 and 18:1 substrates (Tables 3 and 4) indicates that FAE1 is more active when 18:1 is the substrate than when 18:0 is the substrate. In contrast, the overall rate of product formation with EL1 is less when 18:1 is the substrate than when 18:0 is the substrate (Tables 3 and 4). EL2 is also less active when 18:1 is the substrate than when 18:0 is the substrate (Tables 3 and 4). These results support the conclusion that EL1 and EL2 encode novel gene products and suggest that EL1 and EL2 have a preference for saturated fatty acids as substrates, whereas the FAE1 gene product has a preference for monounsaturated fatty acids as substrates.

In a second experiment, microsomes were prepared from galactose-induced and from glucose-repressed yeast cultures containing EL1 or EL2 coding sequences. The microsomal preparations were incubated with either 18:0 acyl-CoA or 18:1 acyl-CoA as substrate and the fatty acid reaction products determined as described above. The results with the 18:0 substrate are shown in Table 5. The results with the 18:1 substrate are shown in Table 6.

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Table 5.

Elongation of 18:0-CoA by EL1 and EL2
With and Without Induction of Gene Expression

		ß-Keto Acyl Synthase Gene										
		E	L1		E	L2	-					
Acyl	+Glu	cose	+Gala	+Galactose		cose	+Galactose					
CoA	Rate ¹	(%)	Rate	(%)	Rate	(%)	Rate	(%)				
20:0	0.007	100.0	0.074	55.8	0.030	81.3	0.107	43.1				
22:0	0.000	0.0	0.023	17.4	0.002	5.1	0.044	17.8				
24:0	0.000	0.0	0.020	15.3	0.005	13.6	0.048	19.1				
26:0	0.000	0.0	0.015	11.5	0.000	0.0	0.050	20.0				
Total	0.007	100.0	0.133	100.0	0.037	100.0	0.249	100.0				

Nanomoles/minute/mg of microsomal protein

Table 6.
Elongation of 18:1-CoA by EL1 and EL2
With and Without Induction of Gene Expression

	1							*					
	ß-Keto Acyl Synthase Gene												
		Е	L1				EL2						
Acyl	+Glu	+Glucose +Galactose			+Glu	cose	+Galactose						
CoA	Rate ¹	(%)	Rate	(%)	Rate	(%)	Rate	(%)					
20:1	0.062	100.0	0.081	100.0	0.043	100.0	0.089	100.0					
22:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0					
24:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0					
26:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0					
Total	0.062	100.0	0.081	100.0	0.043	100.0	0.089	100.0					

Nanomoles/minute/mg of microsomal protein

The results in Table 5 show in vitro elongase activity for EL1 and EL2 under induced (galactose) and uninduced (glucose) conditions. The comparison indicates that induction with galactose results in a large increase in overall elongase activity when 18:0 acyl CoA is the substrate (about 19-fold and 7-fold for EL1 and EL2, respectively). In contrast, induction when 18:1 acyl CoA is the substrate results in only a small increase in elongase activity (about 1.3-fold and 2-fold for EL1 and El2, respectively), as shown in Table 6.

The results in Table 5 show that little or no VLCFA products are made by yeast microsomes under uninduced conditions. Upon induction of EL1 and EL2 gene

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expression, however, significant quantities of C20:0, C22:0, C24:0 and C26:0 are made. The data in Tables 5 and 6 are consistent with the results in Tables 3 and 4, which indicated that EL1 and EL2 were more active with a saturated fatty acid substrate than with a monounsaturated substrate.

The data in Tables 5 and 6 are also consistent with the data in Tables 3 and 4 indicating that the EL1 and EL2 gene products are more active in converting C24:0 to C26:0 than is FAE1.

In a third experiment, microsomes from induced and uninduced cultures containing EL1 or EL2 were incubated in the absence of cofactors involved in the ß-ketoacyl condensation reaction. Cultures were induced and microsomes were prepared as described in Example 2. In vitro assays were carried out as described in Example 2, except that either ATP, CoASH or both were omitted from the enzyme reaction mixture. In addition, one reaction was carried out in a complete mixture having 0.01 mM of cerulenin (Sigma, St. Louis, MO). Cerulenin is an inhibitor of some condensing enzymes. The results are shown in Tables 7-9.

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Table 7. Effect of Cofactors on 18:0-CoA Elongation1

Gene	Expt4	+Glu²	+Gal²	-ATP ³	-CoA³	-A&C ³	+ Cer³
EL1	1	.037	.109	.095	.105	.119	.141
	2	N.D.	.090	.125	.093	.270	.176
EL2	1	.033	.112	.168	.127	.143	.238
	2	N.D.	.120	.178	.133	.195	.302

¹ Activity in nanomoles/minute/mg of microsomal protein.

 $^{^{2}}$ +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.

Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

Experiment No.

Table 8.

Effect of Cofactors on Elongation Products of EL1

Prod.	+Glu²	+Gal²	-ATP ³	-CoA³	-A&C ³	+Cer³
20:0	53.9	46.2	34.4	47.8	41.7	46.7
22:0	14.4	18.7	13.7	18.0	19.4	16.2
24:0	18.5	18.1	20.6	19.1	16.7	17.7
26:0	13.2	17.1	31.4	15.2	22.3	19.4
Total	100.0	100.0	100.0	100.0	100.0	100.0

¹ Amount of indicated product as a percent of total products formed. Results of one experiment for +Glucose; Average of two experiments for other conditions.

² +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.

³ Microsomes from galactose-induced cultures. -ATP: ATP omitted from

Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

Table 9.

Effect of Cofactors on Elongation Products of EL2¹

Prod.	+Glu²	+Gal²	-ATP ³	-CoA³	-A&C ³	+Cer³
20:0	54.5	47.1	34.1	45.3	38.0	41.8
22:0	17.1	19.1	16.4	19.2	15.9	16.1
24:0	5.8	19.4	20.8	19.9	18.4	20.4
26:0	22.6	14.5	28.9	15.8	27.8	21.8
Total	100.0	100.0	100.0	100.0	100.0	100.0

¹ Amount of indicated product as a percent of total products formed. Results of one experiment for +Glucose; Average of two experiments for other conditions.

The results in Table 7 indicate that omission of ATP and/or CoA from the incubation mixture does not have a significant effect on the overall amounts of VLCFAs synthesized by the *in vitro* KAS activity of EL1 or EL2. The results also show that cerulenin does not inhibit the KAS activity of EL1 or EL2. The data in Table 8 and 9 confirm that EL1 and EL2 KAS activity produces significant amounts of C24:0 and C26:0 acyl CoA products.

To the extent not already indicated, it will be understood by those of ordinary skill in the art that any one of the various specific embodiments herein described and illustrated may be further modified to incorporate features—shown in other of the specific embodiments.

The foregoing detailed description has been provided for a better understanding of the invention only and no unnecessary limitation should be understood therefrom as some modifications will be apparent to those

² +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.

³ Microsomes from galactose-induced cultures -ATP: ATP omitted from

³ Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

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skilled in the art without deviating from the spirit and scope of the appended claims.

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SEQUENCE LISTING

- (1) GENERAL INFORMATION
- (i) APPLICANT: CARGILL, INCORPORATED
- (ii) TITLE OF THE INVENTION: FATTY ACID ELONGASES
- (iii) NUMBER OF SEQUENCES: 14
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Fish & Richardson P.C., P.A.
 - (B) STREET: 60 South Sixth Street, Suite 3300
 - (C) CITY: Minneapolis
 - (D) STATE: MN
 - (E) COUNTRY: USA
 - (F) ZIP: 55402
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette
 - (B) COMPUTER: IBM Compatible
 - (C) OPERATING SYSTEM: DOS
 - (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/868,373
 - (B) FILING DATE: 03-JUN-1997
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Lundquist, Ronald C
 - (B) REGISTRATION NUMBER: 37,875
 - (C) REFERENCE/DOCKET NUMBER: 07039/064W01
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 612-335-5050
 - (B) TELEFAX: 612-288-9696
 - (C) TELEX:
 - (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1560 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Genomic DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGATCGAG	AGAGATTAAC	GGCGGAGATG	GCGTTTCGAG	ATTCATCATC	GGCCGTTATA	60
AGAATTCGAA	GACGTTTGCC	GGATTTATTA	ACGTCCGTTA	AGCTCAAATA	CGTGAAGCTT	120
GGACTTCACA	ACTCTTGCAA	CGTGACCACC	ATTCTCTTCT	TCTTAATTAT	TCTTCCTTTA	180
ACCGGAACCG	TGCTGGTTCA	GCTAACCGGT	CTAACGTTCG	ATACGTTCTC	TGAGCTTTGG	240
TCTAACCAGG	CGGTTCAACT	CGACACGGCG	ACGAGACTTA	CCTGCTTGGT	TTTCCTCTCC	300
TTCGTTTTGA	CCCTCTACGT	GGCTAACCGG	TCTAAACCGG	TTTACCTAGT	GGATTTCTCC	360
TGCTACAAAC	CGGAAGACGA	GCGTAAAATA	TCAGTAGATT	CGTTCTTGAC	GATGACTGAG	420
GAAAATGGAT	CATTCACCGA	TGACACGGTT	CAGTTCCAGC	AAAGAATCTC	GAACCGGGCC	480

GGTTTGGGAG A	CGAGACGTA	TCTGCCACGT	GGCATAACTT	CAACGCCCCC	GAAGCTAAAT	540
ATGTCAGAGG C	ACGTGCCGA	AGCTGAAGCC	GTTATGTTTG	GAGCCTTAGA	TTCCCTCTTC	600
GAGAAAACCG G	AATTAAACC	GGCCGAAGTC	GGAATCTTGA	TAGTAAACTG	CAGCTTATTC	660
AATCCGACGC C	GTCTCTATC	AGCGATGATC	GTGAACCATT	ACAAGATGAG	AGAAGACATC	720
AAAAGTTACA A	CCTCGGAGG	AATGGGTTGC	TCCGCCGGAT	TAATCTCAAT	CGATCTCGCT	780
AACAATCTCC T	'CAAAGCAAA	CCCTAATTCT	TACGCTGTCG	TGGTAAGCAC	GGAAAACATA	840
ACCCTAAACT G	GTACTTCGG	AAATGACCGG	TCAATGCTCC	TCTGCAACTG	CATCTTCCGA	900
ATGGGCGGAG C	TGCGATTCT	CCTCTCTAAC	CGCCGTCAAG	ACCGGAAGAA	GTCAAAGTAC	960
TCGCTGGTCA A	CGTCGTTCG	AACACATAAA	GGATCAGACG	ACAAGAACTA	CAATTGCGTG	1020
TACCAGAAGG A	AGACGAGAG	AGGAACAATC	GGTGTCTCTT	TAGCTAGAGA	GCTCATGTCT	1080
GTCGCCGGAG A	CGCTCTGAA	AACAAACATC	ACGACTTTAG	GACCGATGGT	TCTTCCATTG	1140
TCAGAGCAGT T	'GATGTTCTT	GATTTCCTTG	GTCAAAAGGA	AGATGTTCAA	GTTAAAAGTT	1200
AAACCGTATA T	TCCGGATTT	CAAGCTAGCT	TTCGAGCATT	TCTGTATTCA	CGCAGGAGGT	1260
AGAGCGGTTC T.	'AGACGAAGT	GCAGAAGAAT	CTTGATCTCA	AAGATTGGCA	CATGGAACCT	1320
TCTAGAATGA C	TTTGCACAG	ATTTGGTAAC	ACTTCGAGTA	GCTCGCTTTG	GTATGAGATG	1380
GCTTATACCG A	AGCTAAGGG	TCGGGTTAAA	GCTGGTGACC	GACTTTGGCA	GATTGCGTTT	1440
GGATCGGGTT T	'CAAGTGTAA	TAGTGCGGTT	TGGAAAGCGT	TACGACCGGT	TTCGACGGAG	1500
GAGATGACCG G	TAATGCTTG	GGCTGGTTCG	ATTGATCAAT	ATCCGGTTAA	AGTTGTGCAA	1560

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 520 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met 1	Asp	Arg	Glu	Arg 5	Leu	Thr	Ala	Glu	Met 10	Ala	Phe	Arg	Asp	Ser 15	Ser
Ser	Ala	Val	Ile 20	Arg	Ile	Arg	Arg	Arg 25	Leu	Pro	Asp	Leu	Leu 30	Thr	Ser
Val	Lys	Leu 35	Lys	Tyr	Val	Lys	Leu 40	Gly	Leu	His	Asn	Ser 45	Cys	Asn	Val
Thr	Thr 50	Ile	Leu	Phe	Phe	Leu 55	Ile	Ile	Leu	Pro	Leu 60	Thr	Gly	Thr	Val
Leu 65	Val	Gln	Leu	Thr	Gly 70	Leu	Thr	Phe	Asp	Thr 75	Phe	Ser	Glu	Leu	Trp 80
Ser	Asn	Gln	Ala	Val 85	Gln	Leu	Asp	Thr	Ala 90	Thr	Arg	Leu	Thr	Cys 95	Leu
Val	Phe	Leu	Ser 100	Phe	Val	Leu	Thr	Leu 105	Tyr	Val	Ala	Asn	Arg 110	Ser	Lys
Pro	Val	Tyr 115	Leu	Val	Asp	Phe	Ser 120	Cys	Tyr	Lys	Pro	Glu 125	Asp	Glu	Arg
Lys	Ile 130	Ser	Val	qaA	Ser	Phe 135	Leu	Thr	Met	Thr	Glu 140	Glu	Asn	Gly	Ser
Phe 145	Thr	Asp	Asp	Thr	Val 150	Gln	Phe	Gln	Gln	Arg 155	Ile	Ser	Asn	Arg	Ala 160
Gly	Leu	Gly	Asp	Glu 165	Thr	Tyr	Leu	Pro	Arg 170	Gly	Ile	Thr	Ser	Thr 175	Pro
Pro	Lys	Leu	Asn 180	Met	Ser	Glu	Ala	Arg 185	Ala	Glu	Ala	Glu	Ala 190	Val	Met
Phe	Gly	Ala 195	Leu	Asp	Ser	Leu	Phe 200	Glu	Lys	Thr	Gly	Ile 205	Lys	Pro	Ala
	210					215		_			220			Thr	
Ser 225	Leu	Ser	Ala	Met	Ile 230	Val	Asn	His	Tyr	Lys 235	Met	Arg	Glu	Asp	Ile 240
Lys	Ser	Tyr	Asn	Leu 245	Gly	Gly	Met	Gly	Cys 250	Ser	Ala	Gly	Leu	Ile 255	Ser
Ile	Asp	Leu	Ala 260	Asn	Asn	Leu	Leu	Lys 265	Ala	Asn	Pro	Asn	Ser 270	Tyr	Ala
Val	Val	Val 275	Ser	Thr	Glu	Asn	Ile 280	Thr	Leu	Asn	Trp	Tyr 285	Phe	Gly	Asn

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Asp Arg Ser Met Leu Leu Cys Asn Cys Ile Phe Arg Met Gly Gly Ala
                        295
                                            300
Ala Ile Leu Leu Ser Asn Arg Arg Gln Asp Arg Lys Lys Ser Lys Tyr
                    310
                                        315
Ser Leu Val Asn Val Val Arg Thr His Lys Gly Ser Asp Asp Lys Asn
               325
                                   330
Tyr Asn Cys Val Tyr Gln Lys Glu Asp Glu Arg Gly Thr Ile Gly Val
            340
                                345
Ser Leu Ala Arg Glu Leu Met Ser Val Ala Gly Asp Ala Leu Lys Thr
                            360
                                                365
Asn Ile Thr Thr Leu Gly Pro Met Val Leu Pro Leu Ser Glu Gln Leu
                        375
                                            380
Met Phe Leu Ile Ser Leu Val Lys Arg Lys Met Phe Lys Leu Lys Val
                    390
                                        395
Lys Pro Tyr Ile Pro Asp Phe Lys Leu Ala Phe Glu His Phe Cys Ile
               405
                                   410
                                                       415
His Ala Gly Gly Arg Ala Val Leu Asp Glu Val Gln Lys Asn Leu Asp
            420
                                425
                                                   430
Leu Lys Asp Trp His Met Glu Pro Ser Arg Met Thr Leu His Arg Phe
        435
                            440
Gly Asn Thr Ser Ser Ser Leu Trp Tyr Glu Met Ala Tyr Thr Glu
                       455
                                            460
Ala Lys Gly Arg Val Lys Ala Gly Asp Arg Leu Trp Gln Ile Ala Phe
                    470
                                        475
Gly Ser Gly Phe Lys Cys Asn Ser Ala Val Trp Lys Ala Leu Arg Pro
               485
                                   490
Val Ser Thr Glu Glu Met Thr Gly Asn Ala Trp Ala Gly Ser Ile Asp
            500
Gln Tyr Pro Val Lys Val Val Gln
        515
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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1479 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Genomic DNA
- (xi) SEQUENCE DESCRIPTION: SEO ID NO:3:

ATGGATTACC	CCATGAAGAA	GGTAAAAATC	TTTTTCAACT	ACCTCATGGC	GCATCGCTTC	60
AAGCTCTGCT	TCTTACCATT	AATGGTTGCT	ATAGCCGTGG	AGGCGTCTCG	TCTTTCCACA	120
CAAGATCTCC	AAAACTTTTA	CCTCTACTTA	CAAAACAACC	ACACATCTCT	AACCATGTTC	180
TTCCTTTACC	TCGCTCTCGG	GTCGACTCTT	TACCTCATGA	CCCGGCCCAA	ACCCGTTTAT	240
CTCGTTGACT	TTAGCTGCTA	CCTCCCACCG	TCGCATCTCA	AAGCCAGCAC	CCAGAGGATC	300
ATGCAACACG	TAAGGCTTGT	ACGAGAAGCA	GGCGCGTGGA	AGCAAGAGTC	CGATTACTTG	360
ATGGACTTCT	GCGAGAAGAT	TCTAGAACGT	TCCGGTCTAG	GCCAAGAGAC	GTACGTACCC	420
GAAGGTCTTC	AAACTTTGCC	ACTACAACAG	AATTTGGCTG	TATCACGTAT	AGAGACGGAG	480
GAAGTTATTA	TTGGTGCGGT	CGATAATCTG	TTTCGCAACA	CGGGAATAAG	CCCTAGTGAT	540
ATAGGTATAT	TGGTGGTGAA	TTCAAGCACT	TTTAATCCAA	CACCTTCGCT	ATCAAGTATC	600
TTAGTGAATA	AGTTTAAACT	TAGGGATAAT	ATAAAGAGCT	TGAATCTTGG	TGGGATGGGG	660
TGTAGCGCTG	GAGTCATCGC	TATCGATGCG	GCTAAGAGCT	TGTTACAAGT	TCATAGAAAC	720
ACTTATGCTC	TTGTGGTGAG	CACGGAGAAC	ATCACTCAAA	ACTTGTACAT	GGGTAACAAC	780
AAATCAATGT	TGGTTACAAA	CTGTTTGTTC	CGTATAGGTG	GGGCCGCGAT	TTTGCTTTCT	840
AACCGGTCTA	TAGATCGTAA	ACGCGCAAAA	TACGAGCTTG	TTCACACCGT	GCGGGTCCAT	900
ACCGGAGCAG	ATGACCGATC	CTATGAATGT	GCAACTCAAG	AAGAGGATGA	AGATGGCATA	960
GTTGGGGTTT	CCTTGTCAAA	GAATCTACCA	ATGGTAGCTG	CAAGAACCCT	AAAGATCAAT	1020
ATCGCAACTT	TGGGTCCGCT	TGTTCTTCCC	ATAAGCGAGA	AGTTTCACTT	CTTTGTGAGG	1080
TTCGTTAAAA	AGAAGTTTCT	CAACCCCAAG	CTAAAGCATT	ACATTCCGGA	TTTCAAGCTC	1140
GCATTCGAGC	ATTTCTGTAT	CCATGCGGGT	GGTAGAGCGC	TAATTGATGA	GATGGAGAAG	1200
AATCTTCATC	TAACTCCACT	AGACGTTGAG	GCTTCAAGAA	TGACATTACA	CAGGTTTGGT	1260
AATACCTCTT	CGAGCTCCAT	TTGGTACGAG	TTGGCTTACA	CAGAAGCCAA	AGGAAGGATG	1320
ACGAAAGGAG	ATAGGATTTG	GCAGATTGCG	TTGGGGTCAG	GTTTTAAGTG	TAATAGTTCA	1380

1479

GTTTGGGTGG CTCTTCGTAA CGTCAAGCCT TCTACTAATA ATCCTTGGGA ACAGTGTCTA CACAAATATC CAGTTGAGAT CGATATAGAT TTAAAAGAG

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 493 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEO ID NO:4:

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Met Asp Tyr Pro Met Lys Lys Val Lys Ile Phe Phe Asn Tyr Leu Met
                                  10
Ala His Arg Phe Lys Leu Cys Phe Leu Pro Leu Met Val Ala Ile Ala
           20
                               25
Val Glu Ala Ser Arg Leu Ser Thr Gln Asp Leu Gln Asn Phe Tyr Leu
                           40
Tyr Leu Gln Asn Asn His Thr Ser Leu Thr Met Phe Phe Leu Tyr Leu
                       55
                                           60
Ala Leu Gly Ser Thr Leu Tyr Leu Met Thr Arg Pro Lys Pro Val Tyr
                 70
                                    75
Leu Val Asp Phe Ser Cys Tyr Leu Pro Pro Ser His Leu Lys Ala Ser
              85
                                90
Thr Gln Arg Ile Met Gln His Val Arg Leu Val Arg Glu Ala Gly Ala
           100
                               105
Trp Lys Gln Glu Ser Asp Tyr Leu Met Asp Phe Cys Glu Lys Ile Leu
       115
                           120
Glu Arg Ser Gly Leu Gly Gln Glu Thr Tyr Val Pro Glu Gly Leu Gln
                       135
Thr Leu Pro Leu Gln Gln Asn Leu Ala Val Ser Arg Ile Glu Thr Glu
                  150
                                      155
Glu Val Ile Ile Gly Ala Val Asp Asn Leu Phe Arg Asn Thr Gly Ile
               165
                                   1.70
                                                      175
Ser Pro Ser Asp Ile Gly Ile Leu Val Val Asn Ser Ser Thr Phe Asn
           180
                               185
Pro Thr Pro Ser Leu Ser Ser Ile Leu Val Asn Lys Phe Lys Leu Arg
       195
                           200
                                               205
Asp Asn Ile Lys Ser Leu Asn Leu Gly Gly Met Gly Cys Ser Ala Gly
   210
                       215
                                           220
Val Ile Ala Ile Asp Ala Ala Lys Ser Leu Leu Gln Val His Arg Asn
                   230
                                      235
Thr Tyr Ala Leu Val Val Ser Thr Glu Asn Ile Thr Gln Asn Leu Tyr
                                   250
Met Gly Asn Asn Lys Ser Met Leu Val Thr Asn Cys Leu Phe Arg Ile
                               265
                                                  270
Gly Gly Ala Ala Ile Leu Leu Ser Asn Arg Ser Ile Asp Arg Lys Arg
        275
                           280
                                               285
Ala Lys Tyr Glu Leu Val His Thr Val Arg Val His Thr Gly Ala Asp
                     295
                                           300
Asp Arg Ser Tyr Glu Cys Ala Thr Gln Glu Glu Asp Glu Asp Gly Ile
                   310
305
                                       315
Val Gly Val Ser Leu Ser Lys Asn Leu Pro Met Val Ala Ala Arg Thr
               325
                                   330
Leu Lys Ile Asn Ile Ala Thr Leu Gly Pro Leu Val Leu Pro Ile Ser
                               345
Glu Lys Phe His Phe Phe Val Arg Phe Val Lys Lys Phe Leu Asn
       355
                           360
                                               365
Pro Lys Leu Lys His Tyr Ile Pro Asp Phe Lys Leu Ala Phe Glu His
                       375
                                           380
Phe Cys Ile His Ala Gly Gly Arg Ala Leu Ile Asp Glu Met Glu Lys
                  390
                                      395
Asn Leu His Leu Thr Pro Leu Asp Val Glu Ala Ser Arg Met Thr Leu
               405
                                   410
```

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- (2) INFORMATION FOR SEQ ID NO:5:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1512 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Genomic DNA
- (xi) SEQUENCE DESCRIPTION: SEO ID NO:5:

CTACGTCAGG	GTAGAACAAA	GAGTAAACAC	TTAAGCAAAA	CAATTTGTCC	TACTCTTAGG	60
TTATCTCCAA	TGAAGAACTT	AAAGATGGTT	TTCTTCAAGA	TCCTCTTTAT	CTCTTTAATG	120
GCAGGATTAG	CCATGAAAGG	ATCTAAGATC	AACGTAGAAG	ATCTCCAAAA	GTTCTCCCTC	180
CACCATACAC	AGAACAACCT	CCAAACCATA	AGCCTTCTAT	TGTTTCTTGT	CGTTTTTGTG	240
TGGATCCTCT	ACATGTTAAC	CCGACCTAAA	CCCGTTTACC	TTGTTGATTT	CTCCTGCTAC	300
CTTCCACCGT	CGCATCTCAA	GGTCAGTATC	CAAACCCTAA	TGGGACACGC	AAGACGTGCA	360
AGAGAAGCAG	GCATGTGTTG	GAAGAACAAA	GAGAGCGACC	ATTTAGTTGA	CTTCCAGGAG	420
AAGATTCTTG	AACGTTCCGG	TCTTGGTCAA	GAAACCTACA	TCCCCGAGGG	TCTTCAGTGC	480
TTCCCACTTC	AGCAAGGCAT	GGGTGCTTCA	CGTAAAGAGA	CGGAAGAAGT	AATCTTCGGA	540
GCTCTTGACA	ATCTTTTTCG	CAACACCGGT	GTAAAACCTG	ATGATATCGG	TATATTGGTG	600
GTGAATTCTA	GCACGTTTAA	TCCAACTCCA	TCACTCGCCT	CCATGATTGT	GAACAAGTAC	660
AAACTCAGAG	ACAACATCAA	GAGTTTGAAT	CTTGGAGGGA	TGGGTTGCAG	TGCCGGAGTT	720
ATAGCTGTTG	ATGTCGCTAA	GGGATTACTA	CAAGTTCATA	GGAACACTTA	TGCTATTGTA	780
GTAAGCACAG	AGAACATCAC	TCAGAACTTA	TACTTGGGGA	AAAACAAATC	AATGCTAGTC	840
ACAAACTGTT	TGTTCCGCGT	TGGTGGTGCT	GCGGTTCTGC	TTTCAAACAG	ATCTAGAGAC	900
CGTAACCGCG	CCAAATACGA	GCTTGTTCAC	ACCGTACGGA	TCCATACCGG	ATCAGATGAT	960
AGGTCGTTCG	AATGTGCGAC	ACAAGAAGAG	GATGAAGATG	GTATAATTGG	AGTTACCTTG	1020
ACAAAGAATC	TACCTATGGT	GGCTGCAAGG	ACTCTTAAGA	TAAATATCGC	AACTTTGGGT	1080
CCTCTTGTAC	TTCCATTAAA	AGAGAAGCTA	GCCTTCTTTA	TTACTTTTGT	CAAGAAGAAG	1140
TATTTCAAGC	CAGAGTTAAG	GAATTATACA	CCAGATTTCA	AGCTTGCCTT	TGAGCATTTC	1200
TGTATCCACG	CTGGTGGAAG	AGCTCTAATA	GATGAGCTGG	AGAAGAACCT	TAAGCTTTCT	1260
CCGTTACACG	TAGAGGCGTC	AAGAATGACA	CTACACAGGT	TTGGTAACAC	TTCTTCTAGC	1320
TCAATCTGGT	ACGAGTTAGC	TTATACAGAA	GCTAAAGGAA	GGATGAAGGA	AGGAGATAGG	1380
ATTTGGCAGA	TTGCTTTGGG	GTCAGGTTTT	AAGTGTAACA	GTTCAGTATG	GGTGGCTCTG	1440
CGAGACGTTA	AGCCTTCAGC	TAACAGTCCA	TGGGAAGACT	GTATGGATAG	ATATCCGGTT	1500
GAGATTGATA	TT					1512

- (2) INFORMATION FOR SEQ ID NO:6:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 504 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Arg Gln Gly Arg Thr Lys Ser Lys His Leu Ser Lys Thr Ile Cys 1 5 10 15

Pro Thr Leu Arg Leu Ser Pro Met Lys Asn Leu Lys Met Val Phe Phe 20 25 30

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Lys Ile Leu Phe Ile Ser Leu Met Ala Gly Leu Ala Met Lys Gly Ser Lys Ile Asn Val Glu Asp Leu Gln Lys Phe Ser Leu His His Thr Gln Asn Asn Leu Gln Thr Ile Ser Leu Leu Leu Phe Leu Val Val Phe Val Trp Ile Leu Tyr Met Leu Thr Arg Pro Lys Pro Val Tyr Leu Val Asp Phe Ser Cys Tyr Leu Pro Pro Ser His Leu Lys Val Ser Ile Gln Thr 1.00 Leu Met Gly His Ala Arg Arg Ala Arg Glu Ala Gly Met Cys Trp Lys Asn Lys Glu Ser Asp His Leu Val Asp Phe Gln Glu Lys Ile Leu Glu Arg Ser Gly Leu Gly Gln Glu Thr Tyr Ile Pro Glu Gly Leu Gln Cys Phe Pro Leu Gln Gln Gly Met Gly Ala Ser Arg Lys Glu Thr Glu Glu Val Ile Phe Gly Ala Leu Asp Asn Leu Phe Arg Asn Thr Gly Val Lys Pro Asp Asp Ile Gly Ile Leu Val Val Asn Ser Ser Thr Phe Asn Pro Thr Pro Ser Leu Ala Ser Met Ile Val Asn Lys Tyr Lys Leu Arg Asp Asn Ile Lys Ser Leu Asn Leu Gly Gly Met Gly Cys Ser Ala Gly Val Ile Ala Val Asp Val Ala Lys Gly Leu Leu Gln Val His Arg Asn Thr Tyr Ala Ile Val Val Ser Thr Glu Asn Ile Thr Gln Asn Leu Tyr Leu Gly Lys Asn Lys Ser Met Leu Val Thr Asn Cys Leu Phe Arg Val Gly Gly Ala Ala Val Leu Leu Ser Asn Arg Ser Arg Asp Arg Asn Arg Ala Lys Tyr Glu Leu Val His Thr Val Arg Ile His Thr Gly Ser Asp Asp Arg Ser Phe Glu Cys Ala Thr Gln Glu Glu Asp Glu Asp Gly Ile Ile Gly Val Thr Leu Thr Lys Asn Leu Pro Met Val Ala Ala Arg Thr Leu Lys Ile Asn Ile Ala Thr Leu Gly Pro Leu Val Leu Pro Leu Lys Glu Lys Leu Ala Phe Phe Ile Thr Phe Val Lys Lys Lys Tyr Phe Lys Pro Glu Leu Arg Asn Tyr Thr Pro Asp Phe Lys Leu Ala Phe Glu His Phe Cys Ile His Ala Gly Gly Arg Ala Leu Ile Asp Glu Leu Glu Lys Asn Leu Lys Leu Ser Pro Leu His Val Glu Ala Ser Arg Met Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr Glu Leu Ala Tyr Thr Glu Ala Lys Gly Arg Met Lys Glu Gly Asp Arg Ile Trp Gln Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ser Val Trp Val Ala Leu Arg Asp Val Lys Pro Ser Ala Asn Ser Pro Trp Glu Asp Cys Met Asp Arg Tyr Pro Val Glu Ile Asp Ile

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- (2) INFORMATION FOR SEO ID NO:7:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1650 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Genomic DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGGGTAGAT	CCAACGAGCA	AGATCTGCTC	TCTACCGAGA	TCGTTAATCG	TGGGATCGAA	60
CCATCCGGTC	CTAACGCCGG	CTCACCAACG	TTCTCGGTTA	GGGTCAGGAG	ACGTTTGCCT	120
GATTTTCTTC	AGTCGGTGAA	CTTGAAGTAC	GTGAAACTTG	GTTACCACTA	CCTCATAAAC	180
CATGCGGTTT	ATTTGGCGAC	CATACCGGTT	CTTGTGCTGG	TTTTTAGTGC	TGAGGTTGGG	240
AGTTTAAGCA	GAGAAGAGAT	TTGGAAGAAG	CTTTGGGACT	ATGATCTTGC	AACTGTTATC	300
GGATTCTTCG	GTGTCTTTGT	TTTAACCGCT	TGTGTCTACT	TCATGTCTCG	TCCTCGCTCT	360
GTTTATCTTA	TTGATTTCGC	TTGTTACAAG	CCCTCCGATG	AACACAAGGT	GACAAAAGAA	420
GAGTTCATAG	AACTAGCGAG	AAAATCAGGG	AAGTTCGACG	AAGAGACACT	CGGTTTCAAG	480
AAGAGGATCT	TACAAGCCTC	AGGCATAGGC	GACGAGACAT	ACGTCCCAAG	ATCCATCTCT	540
TCATCAGAAA	ACATAACAAC	GATGAAAGAA	GGTCGTGAAG	AAGCCTCTAC	AGTGATCTTT	600
GGAGCACTAG	ACGAACTCTT	CGAGAAGACA	CGTGTAAAAC	CTAAAGACGT	TGGTGTCCTT	660
GTGGTTAACT	GTAGCATTTT	CAACCCGACA	CCGTCGTTGT	CCGCAATGGT	GATAAACCAT	720
TACAAGATGA	GAGGGAACAT	ACTTAGTTAC	AACCTTGGAG	GGATGGGATG	TTCGGCTGGA	780
ATCATAGCTA	TTGATCTTGC	TCGTGACATG	CTTCAGTCTA	ACCCTAATAG	TTATGCTGTT	840
GTTGTGAGTA	CTGAGATGGT	TGGGTATAAT	TGGTACGTGG	GAAGTGACAA	GTCAATGGTT	900
ATACCTAATT	GTTTCTTTAG	GATGGGTTGT	TCTGCCGTTA	TGCTCTCTAA	CCGTCGTCGT	960
GACTTTCGCC	ATGCTAAGTA	CCGTCTCGAG	CACATTGTCC	GAACTCATAA	GGCTGCTGAC	1020
GACCGTAGCT	TCAGGAGTGT	GTACCAGGAA	GAAGATGAAC	AAGGATTCAA	GGGGTTGAAG	1080
ATAAGTAGAG	ACTTAATGGA	AGTTGGAGGT	GAAGCTCTCA	AGACAAACAT	CACTACCTTA	1140
GGTCCTCTTG	TCCTACCTTT	CTCCGAGCAG	CTTCTCTTCT	TTGCTGCTTT	GGTCCGCCGA	1200
ACATTCTCAC	CTGCTGCCAA	AACGTCCACA	ACCACTTCCT	TCTCTACTTC	CGCCACCGCA	1260
AAAACCAATG	GAATCAAGTC	TTCCTCTTCC	GATCTGTCCA	AGCCATACAT	CCCGGACTAC	1320
AAGCTCGCCT	TCGAGCATTT	TTGCTTCCAC	GCGGCAAGCA	AAGTAGTGCT	TGAAGAGCTT	1380
CAAAAGAATC	TAGGCTTGAG	TGAAGAGAAT	ATGGAGGCTT	CTAGGATGAC	ACTTCACAGG	1440
TTTGGAAACA	CTTCTAGCAG	TGGAATCTGG	TATGAGTTGG	CTTACATGGA	GGCCAAGGAA	1500
AGTGTTCGTA	GAGGCGATAG	GGTTTGGCAG	ATCGCTTTCG	GTTCTGGTTT	TAAGTGTAAC	1560
AGTGTGGTGT	GGAAGGCAAT	GAGGAAGGTG	AAGAAGCCAA	CCAGGAACAA	TCCTTGGGTG	1620
GATTGCATCA	ACCGTTACCC	TGTGCCTCTC				1650

- (2) INFORMATION FOR SEQ ID NO:8:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 550 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: None
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Gly Arg Ser Asn Glu Gln Asp Leu Leu Ser Thr Glu Ile Val Asn
                                    10
Arg Gly Ile Glu Pro Ser Gly Pro Asn Ala Gly Ser Pro Thr Phe Ser
            20
                                25
                                                    30
Val Arg Val Arg Arg Arg Leu Pro Asp Phe Leu Gln Ser Val Asn Leu
        35
                            40
Lys Tyr Val Lys Leu Gly Tyr His Tyr Leu Ile Asn His Ala Val Tyr
                        55
    50
                                            60
Leu Ala Thr Ile Pro Val Leu Val Leu Val Phe Ser Ala Glu Val Gly
                    70
                                        75
Ser Leu Ser Arg Glu Glu Ile Trp Lys Lys Leu Trp Asp Tyr Asp Leu
                85
                                    90
                                                        95
Ala Thr Val Ile Gly Phe Phe Gly Val Phe Val Leu Thr Ala Cys Val
                                105
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Tyr Phe Met Ser Arg Pro Arg Ser Val Tyr Leu Ile Asp Phe Ala Cys 120 Tyr Lys Pro Ser Asp Glu His Lys Val Thr Lys Glu Glu Phe Ile Glu 135 Leu Ala Arg Lys Ser Gly Lys Phe Asp Glu Glu Thr Leu Gly Phe Lys 150 155 Lys Arg Ile Leu Gln Ala Ser Gly Ile Gly Asp Glu Thr Tyr Val Pro 165 170 Arg Ser Ile Ser Ser Ser Glu Asn Ile Thr Thr Met Lys Glu Gly Arg 180 1.85 Glu Glu Ala Ser Thr Val Ile Phe Gly Ala Leu Asp Glu Leu Phe Glu 200 195 205 Lys Thr Arg Val Lys Pro Lys Asp Val Gly Val Leu Val Val Asn Cys 215 220 Ser Ile Phe Asn Pro Thr Pro Ser Leu Ser Ala Met Val Ile Asn His 230 235 Tyr Lys Met Arg Gly Asn Ile Leu Ser Tyr Asn Leu Gly Gly Met Gly 245 250 Cys Ser Ala Gly Ile Ile Ala Ile Asp Leu Ala Arg Asp Met Leu Gln 265 Ser Asn Pro Asn Ser Tyr Ala Val Val Ser Thr Glu Met Val Gly 280 285 Tyr Asn Trp Tyr Val Gly Ser Asp Lys Ser Met Val Ile Pro Asn Cys 290 295 300 Phe Phe Arg Met Gly Cys Ser Ala Val Met Leu Ser Asn Arg Arg 310 315 Asp Phe Arg His Ala Lys Tyr Arg Leu Glu His Ile Val Arg Thr His 325 330 Lys Ala Ala Asp Asp Arg Ser Phe Arg Ser Val Tyr Gln Glu Glu Asp 340 345 Glu Gln Gly Phe Lys Gly Leu Lys Ile Ser Arg Asp Leu Met Glu Val 355 360 Gly Gly Glu Ala Leu Lys Thr Asn Ile Thr Thr Leu Gly Pro Leu Val 370 375 Leu Pro Phe Ser Glu Gln Leu Leu Phe Phe Ala Ala Leu Val Arg Arg 390 395 Thr Phe Ser Pro Ala Ala Lys Thr Ser Thr Thr Thr Ser Phe Ser Thr 405 410 Ser Ala Thr Ala Lys Thr Asn Gly Ile Lys Ser Ser Ser Ser Asp Leu 420 425 430 Ser Lys Pro Tyr Ile Pro Asp Tyr Lys Leu Ala Phe Glu His Phe Cys 440 Phe His Ala Ala Ser Lys Val Val Leu Glu Leu Gln Lys Asn Leu 455 460 Gly Leu Ser Glu Glu Asn Met Glu Ala Ser Arg Met Thr Leu His Arg 470 475 Phe Gly Asn Thr Ser Ser Gly Ile Trp Tyr Glu Leu Ala Tyr Met 495 Glu Ala Lys Glu Ser Val Arg Arg Gly Asp Arg Val Trp Gln Ile Ala 505 510 Phe Gly Ser Gly Phe Lys Cys Asn Ser Val Val Trp Lys Ala Met Arg 515 520 525 Lys Val Lys Lys Pro Thr Arg Asn Asn Pro Trp Val Asp Cys Ile Asn 535 530 540 Arg Tyr Pro Val Pro Leu 550

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1611 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCGAGCTACG	TCAGGGCTTT	ТАТАТССАСА	AATTCTCATA	AAGTTTTCAA	TTTTATTCCA	60
TTTTTCTCGG	AAGCCATGGA	AGCTGCTAAT	GAGCCTGTTA	ATGGCGGATC	CGTACAGATC	120
CGAACAGAGA	ACAACGAAAG	ACGAAAGCTT	CCTAATTTCT	TACAAAGCGT	CAACATGAAA	180
TACGTCAAGC	TAGGTTATCA	TTACCTCATT	ACTCATCTCT	TCAAGCTCTG	TTTGGTTCCA	240
TTAATGGCGG	TTTTAGTCAC	AGAGATCTCT	CGATTAACAA	CAGACGATCT	TTACCAGATT	300
TGGCTTCATC	TCCAATACAA	TCTCGTTGCT	TTCATCTTTC	TCTCTGCTTT	AGCTATCTTT	360
GGCTCCACCG	TTTACATCAT	GAGTCGTCCC	AGATCTGTTT	ATCTCGTTGA	TTACTCTTGT	420
TATCTTCCTC	CGGAGAGTCT	TCAGGTTAAG	TATCAGAAGT	TTATGGATCA	TTCTAAGTTG	480
ATTGAAGATT	TCAATGAGTC	ATCTTTAGAG	TTTCAGAGGA	AGATTCTTGA	ACGTTCTGGT	540
TTAGGAGAAG	AGACTTATCT	CCCTGAAGCT	TTACATTGTA	TCCCTCCGAG	GCCTACGATG	600
ATGGCGGCTC	GTGAGGAATC	TGAGCAGGTA	ATGTTTGGTG	CTCTTGATAA	GCTTTTCGAG	660
AATACCAAGA	TTAACCCTAG	GGATATTGGT	GTGTTGGTTG	TGAATTGTAG	CTTGTTTAAT	720
CCTACACCTT	CGTTGTCAGC	TATGATTGTT	AACAAGTATA	AGCTTAGAGG	GAATGTTAAG	780
AGTTTTAACC	TTGGTGGAAT	GGGGTGTAGT	GCTGGTGTTA	TCTCTATCGA	TTTAGCTAAA	840
GATATGTTGC	AAGTTCATAG	GAATACTTAT	GCTGTTGTGG	TTAGTACTGA	GAACATTACT	900
CAGAATTGGT	ATTTTGGGAA	TAAGAAGGCT	ATGTTGATTC	CGAATTGTTT	GTTTCGTGTT	960
GGTGGTTCGG	CGATTTTGTT	GTCGAACAAG	GGGAAAGATC	GTAGACGGTC	TAAGTATAAG	1020
CTTGTTCATA	CCGTTAGGAC	TCATAAAGGA	GCTGTTGAGA	AGGCTTTCAA	CTGTGTTTAC	1080
CAAGAGCAAG	ATGATAATGG	GAAGACCGGG	GTTTCGTTGT	CGAAAGATCT	TATGGCTATA	1140
GCTGGGGAAG	CTCTTAAGGC	GAATATCACT	ACTTTAGGTC	CTTTGGTTCT	TCCTATAAGT	1200
GAGCAGATTC	TGTTTTTCAT	GACTTTGGTT	ACGAAGAAAC	TGTTTAACTC	GAAGCTGAAG	1260
CCGTATATTC	CGGATTTCAA	GCTTGCGTTT	GATCATTTCT	GTATCCATGC	TGGTGGTAGA	1320
GCTGTGATTG	ATGAGCTTGA	GAAGAATCTG	CAGCTTTCGC	AGACTCATGT	CGAGGCATCC	1380
AGAATGACAC	TGCACAGATT	TGGAAACACT	TCTTCGAGCT	CGATTTGGTA	TGAACTGGCT	1440
TACATAGAGG	CTAAAGGTAG	GATGAAGAAA	GGAAACCGGG	TTTGGCAGAT	TGCTTTTGGA	1500
AGTGGGTTTA	AGTGTAACAG	TGCAGTTTGG	GTGGCTCTAA	ACAATGTCAA	GCCTTCGGTT	1560
AGTAGTCCGT	GGGAACACTG	CATCGACCGA	TATCCGGTTA	AGCTCGACTT	GCCIICGGII	1611
WGIWGICCGI	GGGWWCWCIG	CATCGACCGA	TWICCGGIIW	WACTCOWCLL	C	TDTT

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 537 amino acids (B) TYPE: amino acid

 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser Ser Tyr Val Arg Ala Phe Ile Cys Thr Asn Ser His Lys Val Phe Asn Phe Ile Pro Phe Phe Ser Glu Ala Met Glu Ala Ala Asn Glu Pro 20 25 Val Asn Gly Gly Ser Val Gln Ile Arg Thr Glu Asn Asn Glu Arg Arg 40 Lys Leu Pro Asn Phe Leu Gln Ser Val Asn Met Lys Tyr Val Lys Leu 55 60 Gly Tyr His Tyr Leu Ile Thr His Leu Phe Lys Leu Cys Leu Val Pro 75 70 Leu Met Ala Val Leu Val Thr Glu Ile Ser Arg Leu Thr Thr Asp Asp 85 90 Leu Tyr Gln Ile Trp Leu His Leu Gln Tyr Asn Leu Val Ala Phe Ile 105 Phe Leu Ser Ala Leu Ala Ile Phe Gly Ser Thr Val Tyr Ile Met Ser 120 125 Arg Pro Arg Ser Val Tyr Leu Val Asp Tyr Ser Cys Tyr Leu Pro Pro 135 140 Glu Ser Leu Gln Val Lys Tyr Gln Lys Phe Met Asp His Ser Lys Leu 150 155 Ile Glu Asp Phe Asn Glu Ser Ser Leu Glu Phe Gln Arg Lys Ile Leu 165 170 175 Glu Arg Ser Gly Leu Gly Glu Glu Thr Tyr Leu Pro Glu Ala Leu His 180 185

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Cys Ile Pro Pro Arg Pro Thr Met Met Ala Ala Arg Glu Glu Ser Glu 200 Gln Val Met Phe Gly Ala Leu Asp Lys Leu Phe Glu Asn Thr Lys Ile 215 220 Asn Pro Arg Asp Ile Gly Val Leu Val Val Asn Cys Ser Leu Phe Asn 230 235 Pro Thr Pro Ser Leu Ser Ala Met Ile Val Asn Lys Tyr Lys Leu Arg 245 250 Gly Asn Val Lys Ser Phe Asn Leu Gly Gly Met Gly Cys Ser Ala Gly 260 265 270 Val Ile Ser Ile Asp Leu Ala Lys Asp Met Leu Gln Val His Arg Asn 275 280 Thr Tyr Ala Val Val Ser Thr Glu Asn Ile Thr Gln Asn Trp Tyr 295 Phe Gly Asn Lys Lys Ala Met Leu Ile Pro Asn Cys Leu Phe Arg Val 310 315 Gly Gly Ser Ala Ile Leu Leu Ser Asn Lys Gly Lys Asp Arg Arg 325 330 335 Ser Lys Tyr Lys Leu Val His Thr Val Arg Thr His Lys Gly Ala Val 340 345 350 Glu Lys Ala Phe Asn Cys Val Tyr Gln Glu Gln Asp Asp Asn Gly Lys 360 365 Thr Gly Val Ser Leu Ser Lys Asp Leu Met Ala Ile Ala Gly Glu Ala 370 375 380 Leu Lys Ala Asn Ile Thr Thr Leu Gly Pro Leu Val Leu Pro Ile Ser 390 395 Glu Gln Ile Leu Phe Phe Met Thr Leu Val Thr Lys Lys Leu Phe Asn 405 410 Ser Lys Leu Lys Pro Tyr Ile Pro Asp Phe Lys Leu Ala Phe Asp His 420 425 Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Glu Leu Glu Lys 440 435 445 Asn Leu Gln Leu Ser Gln Thr His Val Glu Ala Ser Arg Met Thr Leu 455 460 His Arg Phe Gly Asn Thr Ser Ser Ser Ile Trp Tyr Glu Leu Ala 470 475 Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Arg Val Trp Gln 485 490 495 Ile Ala Phe Gly Ser Gly Phe Lys Cys Asn Ser Ala Val Trp Val Ala 500 505 510 Leu Asn Asn Val Lys Pro Ser Val Ser Ser Pro Trp Glu His Cys Ile 520 515 Asp Arg Tyr Pro Val Lys Leu Asp Phe 535

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1502 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Genomic DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCTCCGACGA	TGCCTCAGGC	ACCGATGCCA	GAGTTCTCTA	GCTCGGTGAA	GCTCAAGTAC	60
GTGAAACTTG	GTTACCAATA	TTTGGTTAAC	CATTTCTTGA	GTTTTCTTTT	GATCCCGATC	120
ATGGCTATTG	TCGCCGTTGA	GCTTCTTCGG	ATGGGTCCTG	AAGAGATCCT	TAATGTTTGG	180
AATTCACTCC	AGTTTGACCT	AGTTCAGGTT	CTATGTTCTT	CCTTCTTTGT	CATCTTCATC	240
TCCACTGTTT	ACTTCATGTC	CAAGCCACGC	ACCATCTACC	TCGTTGACTA	TTCTTGTTAC	300
AAGCCACCTG	TCACGTGTCG	TGTCCCCTTC	GCAACTTTCA	TGGAACACTC	TCGTTTGATC	360
CTCAAGGACA	AGCCTAAGAG	CGTCGAGTTC	CAAATGAGAA	TCCTTGAACG	TTCTGGCCTC	420
GGTGAGGAGA	CTTGTCTCCC	TCCGGCTATT	CATTATATTC	CTCCCACACC	AACCATGGAC	480
GCGGCTAGAA	GCGAGGCTCA	GATGGTTATC	TTCGAGGCCA	TGGACGATCT	TTTCAAGAAA	540
ACCGGTCTTA	AACCTAAAGA	CGTCGACATC	CTTATCGTCA	ACTGCTCTCT	TTTCTCTCCC	600

ACACCATCGC	TCTCAGCTAT	GGTCATCAAC	AAATATAAGC	TTAGGAGTAA	TATCAAGAGC	660
TTCAATCTTT	CGGGGATGGG	CTGCAGCGCG	GGCCTGATCT	CAGTTGATCT	AGCCCGCGAC	720
TTGCTCCAAG	TTCATCCCAA	TTCAAATGCA	ATCATCGTCA	GCACGGAGAT	CATAACGCCT	780
AATTACTATC	AAGGCAACGA	GAGAGCCATG	TTGTTACCCA	ATTGTCTCTT	CCGCATGGGT	840
GCGGCAGCCA	TACACATGTC	AAACCGCCGG	TCTGACCGGT	GGCGAGCCAA	ATACAAGCTT	900
TCCCACCTCG	TCCGGACACA	CCGTGGCGCT	GACGACAAGT	CTTTCTACTG	TGTCTACGAA	960
CAGGAAGACA	AAGAAGGACA	CGTTGGCATC	AACTTGTCCA	AAGATCTCAT	GGCCATCGCC	1020
GGTGAAGCCC	TCAAGGCAAA	CATCACCACA	ATAGGTCCTT	TGGTCCTACC	GGCGTCAGAA	1080
CAACTTCTCT	TCCTCACGTC	CCTAATCGGA	CGTAAAATCT	TCAACCCGAA	ATGGAAACCA	1140
TACATACCGG	ATTTCAAGCT	GGCCTTCGAA	CACTTTTGCA	TTCACGCAGG	AGGCAGAGCG	1200
GTGATCGACG	AGCTCCAAAA	GAATCTACAA	CTATCAGGAG	AACACGTTGA	GGCCTCAAGA	1260
ATGACACTAC	ATCGTTTTGG	TAACACGTCA	TCTTCATCGT	TATGGTACGA	GCTTAGCTAC	1320
ATCGAGTCTA	AAGGGAGAAT	GAGGAGAGGC	GATCGCGTTT	GGCAAATCGC	GTTTGGGAGT	1380
GGTTTCAAGT	GTAACTCTGC	CGTGTGGAAG	TGTAACCGTA	CGATTAAGAC	ACCTAAGGAC	1440
GGACCATGGT	CCGATTGTAT	CGACCGTTAC	CCTGTCTTTA	TTCCCGAAGT	TGTCAAACTC	1500
TA						1502

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 500 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Pro Thr Met Pro Gln Ala Pro Met Pro Glu Phe Ser Ser Val Lys Leu Lys Tyr Val Lys Leu Gly Tyr Gln Tyr Leu Val Asn His Phe Leu Ser Phe Leu Leu Ile Pro Ile Met Ala Ile Val Ala Val Glu Leu Leu Arg Met Gly Pro Glu Glu Ile Leu Asn Val Trp Asn Ser Leu Gln Phe Asp Leu Val Gln Val Leu Cys Ser Ser Phe Phe Val Ile Phe Ile Ser Thr Val Tyr Phe Met Ser Lys Pro Arg Thr Ile Tyr Leu Val Asp Tyr Ser Cys Tyr Lys Pro Pro Val Thr Cys Arg Val Pro Phe Ala Thr Phe Met Glu His Ser Arg Leu Ile Leu Lys Asp Lys Pro Lys Ser Val Glu Phe Gln Met Arg Ile Leu Glu Arg Ser Gly Leu Gly Glu Glu Thr Cys Leu Pro Pro Ala Ile His Tyr Ile Pro Pro Thr Pro Thr Met Asp Ala Ala Arg Ser Glu Ala Gln Met Val Ile Phe Glu Ala Met Asp Asp Leu Phe Lys Lys Thr Gly Leu Lys Pro Lys Asp Val Asp Ile Leu Ile Val Asn Cys Ser Leu Phe Ser Pro Thr Pro Ser Leu Ser Ala Met Val Ile Asn Lys Tyr Lys Leu Arg Ser Asn Ile Lys Ser Phe Asn Leu Ser Gly Met Gly Cys Ser Ala Gly Leu Ile Ser Val Asp Leu Ala Arg Asp Leu Leu Gln Val His Pro Asn Ser Asn Ala Ile Ile Val Ser Thr Glu Ile Ile Thr Pro Asn Tyr Tyr Gln Gly Asn Glu Arg Ala Met Leu Leu Pro Asn Cys Leu Phe Arg Met Gly Ala Ala Ile His Met Ser Asn Arg Arg Ser Asp Arg Trp Arg Ala Lys Tyr Lys Leu Ser His Leu Val

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Arg Thr His Arg Gly Ala Asp Asp Lys Ser Phe Tyr Cys Val Tyr Glu
                  310
                                     315
Gln Glu Asp Lys Glu Gly His Val Gly Ile Asn Leu Ser Lys Asp Leu
               325
                                 330
                                                    335
Met Ala Ile Ala Gly Glu Ala Leu Lys Ala Asn Ile Thr Thr Ile Gly
           340
                             345
                                                350
Pro Leu Val Leu Pro Ala Ser Glu Gln Leu Leu Phe Leu Thr Ser Leu
                          360
Ile Gly Arg Lys Ile Phe Asn Pro Lys Trp Lys Pro Tyr Ile Pro Asp
                   375
Phe Lys Leu Ala Phe Glu His Phe Cys Ile His Ala Gly Gly Arg Ala
                  390
                                     395
Val Ile Asp Glu Leu Gln Lys Asn Leu Gln Leu Ser Gly Glu His Val
               405
                                 410
Glu Ala Ser Arg Met Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser
                             425
                                                430
           420
Ser Leu Trp Tyr Glu Leu Ser Tyr Ile Glu Ser Lys Gly Arg Met Arg
                         440
                                   445
       435
Arg Gly Asp Arg Val Trp Gln Ile Ala Phe Gly Ser Gly Phe Lys Cys
                     455
                                        460
Asn Ser Ala Val Trp Lys Cys Asn Arg Thr Ile Lys Thr Pro Lys Asp
                  470
                                  475
Gly Pro Trp Ser Asp Cys Ile Asp Arg Tyr Pro Val Phe Ile Pro Glu
                                  490
Val Val Lys Leu
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(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1548 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Genomic DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

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ATGGACGGTG CCGGAGAATC ACGACTCGGT GGTGATGGTG GTGGTGATGG TTCTGTTGGA
GTTCAGATCC GACAAACACG GATGCTACCG GATTTTCTCC AGAGCGTGAA TCTCAAGTAT
                                                                                         120
GTGAAATTAG GTTACCATTA CTTAATCTCA AATCTCTTGA CTCTCTGTTT ATTCCCTCTC
GCCGTTGTTA TCTCCGTCGA AGCCTCTCAG ATGAACCCAG ATGATCTCAA ACAGCTCTGG
ATCCATCTAC AATACAATCT GGTTAGTATC ATCATCTGTT CAGCGATTCT AGTCTTCGGG TTAACGGTTT ATGTTATGAC CCGACCTAGA CCCGTTTACT TGGTTGATTT CTCTTGTTAT
                                                                                         300
                                                                                         360
CTCCCACCTG ATCATCTCAA AGCTCCTTAC GCTCGGTTCA TGGAACATTC TAGACTCACC
                                                                                         420
GGAGATTTCG ATGACTCTGC TCTCGAGTTT CAACGCAAGA TCCTTGAGCG TTCTGGTTTA
                                                                                         480
GGGGAAGACA CTTATGTCCC TGAAGCTATG CATTATGTTC CACCGAGAAT TTCAATGGCT
                                                                                         540
GCTGCTAGAG AAGAAGCTGA ACAAGTCATG TTTGGTGCTT TAGATAACCT TTTCGCTAAC ACTAATGTGA AACCAAAGGA TATTGGAATC CTTGTTGTGA ATTGTAGTCT CTTTAATCCA
                                                                                         600
                                                                                         660
ACTCCTTCGT TATCTGCAAT GATTGTGAAC AAGTATAAGC TTAGAGGTAA CATTAGAAGC
                                                                                         720
TACAATCTAG GCGGTATGGG TTGCAGCGCG GGAGTTATCG CTGTGGATCT TGCTAAAGAC
ATGTTGTTGG TACATAGGAA CACTTATGCG GTTGTTGTTT CTACTGAGAA CATTACTCAG
                                                                                         840
AATTGGTATT TTGGTAACAA GAAATCGATG TTGATACCGA ACTGCTTGTT TCGAGTTGGT
GGCTCTGCGG TTTTGCTATC GAACAAGTCG AGGGACAAGA GACGGTCTAA GTACAGGCTT
                                                                                         900
                                                                                         960
GTACATGTAG TCAGGACTCA CCGTGGAGCA GATGATAAAG CTTTCCGTTG TGTTTATCAA
                                                                                        1020
GAGCAGGATG ATACAGGGAG AACCGGGGTT TCGTTGTCGA AAGATCTAAT GGCGATTGCA
                                                                                        1080
GGGGAAACTC TCAAAACCAA TATCACTACA TTGGGTCCTC TTGTTCTACC GATAAGTGAG CAGATTCTCT TCTTTATGAC TCTAGTTGTG AAGAAGCTCT TTAACGGTAA AGTGAAACCG TATATCCCGG ATTTCAAACT TGCTTTCGAG CATTTCTGTA TCCATGCTGG TGGAAGAGCT
                                                                                        1140
                                                                                        1200
                                                                                        1260
GTGATCGATG AGTTAGAGAA GAATCTGCAG CTTTCACCAG TTCATGTCGA GGCTTCGAGG
ATGACTCTTC ATCGATTTGG TAACACATCT TCGAGCTCCA TTTGGTATGA ATTGGCTTAC
ATTGAAGCGA AGGGAAGGAT GCGAAGAGGT AATCGTGTTT GGCAAATCGC GTTCGGAAGT GGATTTAAAT GTAATAGCGC GATTTGGGAA GCATTAAGGC ATGTGAAACC TTCGAACAAC
                                                                                        1440
                                                                                        1500
AGTCCTTGGG AAGATTGTAT TGACAAGTAT CCGGTAACTT TAAGTTAT
                                                                                        1548
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(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 516 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Asp Gly Ala Gly Glu Ser Arg Leu Gly Gly Asp Gly Gly Asp Gly Ser Val Gly Val Gln Ile Arg Gln Thr Arg Met Leu Pro Asp Phe 20 25 Leu Gln Ser Val Asn Leu Lys Tyr Val Lys Leu Gly Tyr His Tyr Leu 40 Ile Ser Asn Leu Leu Thr Leu Cys Leu Phe Pro Leu Ala Val Val Ile 55 Ser Val Glu Ala Ser Gln Met Asn Pro Asp Asp Leu Lys Gln Leu Trp Ile His Leu Gln Tyr Asn Leu Val Ser Ile Ile Ile Cys Ser Ala Ile 90 Leu Val Phe Gly Leu Thr Val Tyr Val Met Thr Arg Pro Arg Pro Val 100 105 110 Tyr Leu Val Asp Phe Ser Cys Tyr Leu Pro Pro Asp His Leu Lys Ala 120 125 Pro Tyr Ala Arg Phe Met Glu His Ser Arg Leu Thr Gly Asp Phe Asp 135 140 Asp Ser Ala Leu Glu Phe Gln Arg Lys Ile Leu Glu Arg Ser Gly Leu 150 155 Gly Glu Asp Thr Tyr Val Pro Glu Ala Met His Tyr Val Pro Pro Arg 165 170 Ile Ser Met Ala Ala Ala Arg Glu Glu Ala Glu Gln Val Met Phe Gly 185 Ala Leu Asp Asn Leu Phe Ala Asn Thr Asn Val Lys Pro Lys Asp Ile 195 200 205 Gly Ile Leu Val Val Asn Cys Ser Leu Phe Asn Pro Thr Pro Ser Leu 215 220 Ser Ala Met Ile Val Asn Lys Tyr Lys Leu Arg Gly Asn Ile Arg Ser 230 235 Tyr Asn Leu Gly Gly Met Gly Cys Ser Ala Gly Val Ile Ala Val Asp 245 250 Leu Ala Lys Asp Met Leu Leu Val His Arg Asn Thr Tyr Ala Val Val 265 Val Ser Thr Glu Asn Ile Thr Gln Asn Trp Tyr Phe Gly Asn Lys 280 285 Ser Met Leu Ile 'Pro Asn Cys Leu Phe Arg Val Gly Gly Ser Ala Val 295 300 Leu Leu Ser Asn Lys Ser Arg Asp Lys Arg Arg Ser Lys Tyr Arg Leu 310 315 Val His Val Val Arg Thr His Arg Gly Ala Asp Asp Lys Ala Phe Arg 325 330 Cys Val Tyr Gln Glu Gln Asp Asp Thr Gly Arg Thr Gly Val Ser Leu 345 340 350 Ser Lys Asp Leu Met Ala Ile Ala Gly Glu Thr Leu Lys Thr Asn Ile 360 365 Thr Thr Leu Gly Pro Leu Val Leu Pro Ile Ser Glu Gln Ile Leu Phe 375 Phe Met Thr Leu Val Val Lys Lys Leu Phe Asn Gly Lys Val Lys Pro 390 395 Tyr Ile Pro Asp Phe Lys Leu Ala Phe Glu His Phe Cys Ile His Ala 405 410 Gly Gly Arg Ala Val Ile Asp Glu Leu Glu Lys Asn Leu Gln Leu Ser 420 425 430 Pro Val His Val Glu Ala Ser Arg Met Thr Leu His Arg Phe Gly Asn 440

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WHAT IS CLAIMED IS:

- 1. An isolated polynucleotide encoding a polypeptide having an amino acid sequence selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an amino acid sequence substantially identical to SEQ ID NO:12, and an amino acid sequence substantially identical to SEQ ID NO:14.
 - 2. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:2.
 - 3. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:4.
 - 4. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:6.
 - 5. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:8.
 - 6. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:10.
 - 7. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:12.
 - 8. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:14.

- 9. An isolated polynucleotide, wherein said polynucleotide is selected from the group consisting of:
 - a) SEQ ID NO:1;
 - b) SEQ ID NO:3;
 - c) SEQ ID NO:5;
 - d) SEQ ID NO:7;
 - e) SEQ ID NO:9;
 - f) SEQ ID NO:11;
 - g) SEQ ID NO:13;
 - h) an RNA analog of SEQ ID NO:1;
 - i) an RNA analog of SEQ ID NO:3;
 - j) an RNA analog of SEQ ID NO:5;
 - k) an RNA analog of SEQ ID NO:7;
 - 1) an RNA analog of SEQ ID NO:9;
 - m) an RNA analog of SEQ ID NO:11;
 - n) an RNA analog of SEQ ID NO:13;
- o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k, l), m), or n); and
- p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.
- 10. An isolated polypeptide having an amino acid sequence selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an

amino acid sequence substantially identical to SEQ ID NO:14.

- 11. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:2.
- 12. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:4.
- 13. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:6.
- 14. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:8.
- 15. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:10.
- 16. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:12.
- 17. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:14.
- 18. A transgenic plant containing a nucleic acid construct comprising a polynucleotide selected from the group consisting of:
 - a) SEQ ID NO:1;
 - b) SEQ ID NO:3;
 - c) SEQ ID NO:5;
 - d) SEQ ID NO:7;
 - e) SEQ ID NO:9;
 - f) SEQ ID NO:11;
 - g) SEQ ID NO:13;
 - h) an RNA analog of SEQ ID NO:1;

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- i) an RNA analog of SEQ ID NO:3;
- j) an RNA analog of SEQ ID NO:5;
- k) an RNA analog of SEQ ID NO:7;
- 1) an RNA analog of SEQ ID NO:9;
- m) an RNA analog of SEQ ID NO:11;
- n) an RNA analog of SEQ ID NO:13;
- o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k), l), m), or n); and
- p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.
- 19. The plant of claim 18, wherein said construct further comprises a regulatory element operably linked to said polynucleotide.
- 20. The plant of claim 19, wherein said regulatory element is a tissue-specific promoter.
- 21. The plant of claim 20, wherein said regulatory element is an epidermal cell-specific promoter.
- 22. The plant of claim 20, wherein said regulatory element is a seed-specific promoter that is operably linked in sense orientation to said polynucleotide.
- 23. The plant of claim 22, wherein said plant has altered levels of very long chain fatty acids in seeds compared to the levels in a plant lacking said nucleic acid construct.

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- 24. A transgenic plant containing a nucleic acid construct comprising a polynucleotide encoding a polypeptide selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an amino acid sequence substantially identical to SEQ ID NO:14.
- 25. The plant of claim 24, wherein said construct further comprises a regulatory element operably linked to said polynucleotide.
- 26. The plant of claim 25, wherein said regulatory element is a tissue-specific promoter.
- 27. The plant of claim 26, wherein said regulatory element is an epidermal cell-specific promoter.
- 28. The plant of claim 26, wherein said regulatory element is a seed-specific promoter that is operably linked in sense orientation to said polynucleotide.
- 29. The plant of claim 28, wherein said plant has altered levels of very long chain fatty acids in seeds compared to the levels in a plant lacking said nucleic acid construct.
- 30. A method of altering the levels of very long chain fatty acids in a plant, comprising the steps of:

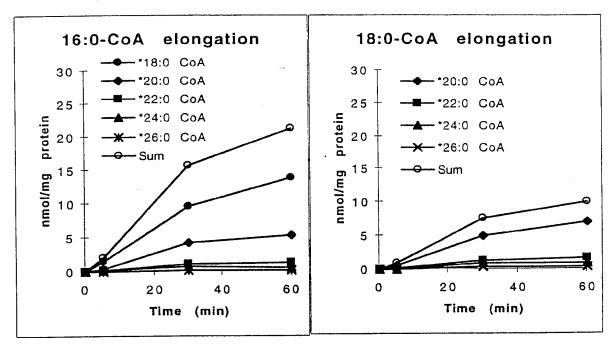
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A) creating a nucleic acid construct, said construct comprising a polynucleotide selected from the group consisting of:

```
a) SEQ ID NO:1;
```

- b) SEQ ID NO:3;
- c) SEQ ID NO:5;
- d) SEQ ID NO:7;
- e) SEQ ID NO:9;
- f) SEQ ID NO:11;
- q) SEQ ID NO:13;
- h) an RNA analog of SEQ ID NO:1;
- i) an RNA analog of SEQ ID NO:3;
- j) an RNA analog of SEQ ID NO:5;
- k) an RNA analog of SEQ ID NO:7;
- 1) an RNA analog of SEQ ID NO:9;
- m) an RNA analog of SEQ ID NO:11;
- n) an RNA analog of SEQ ID NO:13;
- o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k, l), m), or n); and
- p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14; and B) introducing said construct into said plant, wherein said polynucleotide is effective for altering the levels of very long chain fatty acids in said plant.

Figure / FAE1 w/ respect to time



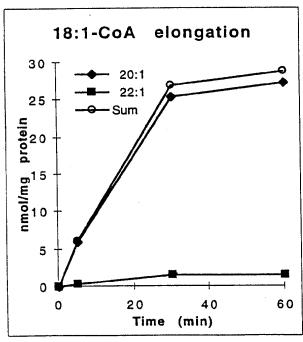
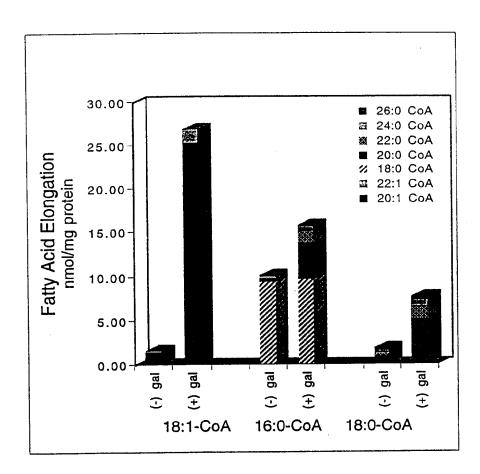


Figure 2



EL1 1560	bases				
ATGGATCGAG	AGAGATTAAC	GGCGGAGATG	GCGTTTCGAG	ATTCATCATC	GGCCGTTATA
AGAATTCGAA	GACGTTTGCC	GGATTTATTA	ACGTCCGTTA	AGCTCAAATA	CGTGAAGCTT
GGACTTCACA	ACTCTTGCAA	CGTGACCACC	ATTCTCTTCT	TCTTAATTAT	TCTTCCTTTA
ACCGGAACCG	TGCTGGTTCA	GCTAACCGGT	CTAACGTTCG	ATACGTTCTC	TGAGCTTTGG
TCTAACCAGG	CGGTTCAACT	CGACACGGCG	ACGAGACTTA	CCTGCTTGGT	TTTCCTCTCC
TTCGTTTTGA	CCCTCTACGT	GGCTAACCGG	TCTAAACCGG	TTTACCTAGT	GGATTTCTCC
TGCTACAAAC	CGGAAGACGA	GCGTAAAATA	TCAGTAGATT	CGTTCTTGAC	GATGACTGAG
GAAAATGGAT	CATTCACCGA	TGACACGGTT	CAGTTCCAGC	AAAGAATCTC	GAACCGGGCC
GGTTTGGGAG	ACGAGACGTA	TCTGCCACGT	GGCATAACTT	CAACGCCCCC	GAAGCTAAAT
ATGTCAGAGG	CACGTGCCGA	AGCTGAAGCC	GTTATGTTTG	GAGCCTTAGA	TTCCCTCTTC
GAGAAAACCG	GAATTAAACC	GGCCGAAGTC	GGAATCTTGA	TAGTAAACTG	CAGCTTATTC
AATCCGACGC	CGTCTCTATC	AGCGATGATC	GTGAACCATT	ACAAGATGAG	AGAAGACATC
AAAAGTTACA	ACCTCGGAGG	AATGGGTTGC	TCCGCCGGAT	TAATCTCAAT	CGATCTCGCT
AACAATCTCC	TCAAAGCAAA	CCCTAATTCT	TACGCTGTCG	TGGTAAGCAC	GGAAAACATA
ACCCTAAACT	GGTACTTCGG	AAATGACCGG	TCAATGCTCC	TCTGCAACTG	CATCTTCCGA
ATGGGCGGAG	CTGCGATTCT	CCTCTCTAAC	CGCCGTCAAG	ACCGGAAGAA	GTCAAAGTAC
TCGCTGGTCA	ACGTCGTTCG	AACACATAAA	GGATCAGACG	ACAAGAACTA	CAATTGCGTG
TACCAGAAGG	AAGACGAGAG	AGGAACAATC	GGTGTCTCTT	TAGCTAGAGA	GCTCATGTCT
GTCGCCGGAG	ACGCTCTGAA	AACAAACATC	ACGACTTTAG	GACCGATGGT	TCTTCCATTG
TCAGAGCAGT	TGATGTTCTT	GATTTCCTTG	GTCAAAAGGA	AGATGTTCAA	GTTAAAAGTT
AAACCGTATA	TTCCGGATTT	CAAGCTAGCT	TTCGAGCATT	TCTGTATTCA	CGCAGGAGGT
AGAGCGGTTC	TAGACGAAGT	GCAGAAGAAT	CTTGATCTCA	AAGATTGGCA	CATGGAACCT
TCTAGAATGA	CTTTGCACAG	ATTTGGTAAC	ACTTCGAGTA	GCTCGCTTTG	GTATGAGATG
GCTTATACCG	AAGCTAAGGG	TCGGGTTAAA	GCTGGTGACC	GACTTTGGCA	GATTGCGTTT
GGATCGGGTT	TCAAGTGTAA	TAGTGCGGTT	TGGAAAGCGT	TACGACCGGT	TTCGACGGAG
GAGATGACCG	GTAATGCTTG	GGCTGGTTCG	ATTGATCAAT	ATCCGGTTAA	AGTTGTGCAA

EL1 FIGURE 3

EL1 sequence
Molecular Weight 58379.00 Daltons
520 Amino Acids
62 Strongly Basic(+) Amino Acids (K,R)
52 Strongly Acidic(-) Amino Acids (D,E)
187 Hydrophobic Amino Acids (A,I,L,F,W,V)
144 Polar Amino Acids (N,C,Q,S,T,Y)
8.784 Isolectric Point
10.804 Charge at PH 7.0

MDRERLTAEM	AFRDSSSAVI	RIRRRLPDLL	TSVKLKYVKL	GLHNSCNVTT	ILFFLIILPL
TGTVLVQLTG	LTFDTFSELW	SNQAVQLDTA	TRLTCLVFLS	FVLTLYVANR	SKPVYLVDFS
CYKPEDERKI					
				NPTPSLSAMI	
				TLNWYFGNDR	
				YQKEDERGTI	
				KPYIPDFKLA	
RAVLDEVQKN	LDLKDWHMEP	SRMTLHRFGN	TSSSSLWYEM	AYTEAKGRVK	AGDRLWQIAF
CCCEKCNCAU	WKALRPUSTE	EMTGNAWAGS	TDOYPVKVVO		

FIGURE 4

EL2 1	479 bases					
ATGGATTACC	CCATGAAGAA	GGTAAAAATC	TTTTTCAACT	ACCTCATGGC	GCATCGCTTC	
AAGCTCTGCT	TCTTACCATT	AATGGTTGCT	ATAGCCGTGG	AGGCGTCTCG	TCTTTCCACA	120
CAAGATCTCC	AAAACTTTTA	CCTCTACTTA	CAAAACAACC	ACACATCTCT	AACCATGTTC	
TTCCTTTACC	TCGCTCTCGG	GTCGACTCTT	TACCTCATGA	CCCGGCCCAA	ACCCGTTTAT	240
CTCGTTGACT	TTAGCTGCTA	CCTCCCACCG	TCGCATCTCA	AAGCCAGCAC	CCAGAGGATC	
ATGCAACACG	TAAGGCTTGT	ACGAGAAGCA	GGCGCGTGGA	AGCAAGAGTC	CGATTACTTG	360
ATGGACTTCT	GCGAGAAGAT	TCTAGAACGT	TCCGGTCTAG	GCCAAGAGAC	GTACGTACCC	
GAAGGTCTTC	AAACTTTGCC	ACTACAACAG	AATTTGGCTG	TATCACGTAT	AGAGACGGAG	480
GAAGTTATTA	TTGGTGCGGT	CGATAATCTG	TTTCGCAACA	CGGGAATAAG	CCCTAGTGAT	
ATAGGTATAT	TGGTGGTGAA	TTCAAGCACT	TTTAATCCAA	CACCTTCGCT	ATCAAGTATC	600
TTAGTGAATA	AGTTTAAACT	TAGGGATAAT	ATAAAGAGCT	TGAATCTTGG	TGGGATGGGG	
TGTAGCGCTG	GAGTCATCGC	TATCGATGCG	GCTAAGAGCT	TGTTACAAGT	TCATAGAAAC	720
ACTTATGCTC	TTGTGGTGAG	CACGGAGAAC	ATCACTCAAA	ACTTGTACAT	GGGTAACAAC	
AAATCAATGT	TGGTTACAAA	CTGTTTGTTC	CGTATAGGTG	GGGCCGCGAT	TTTGCTTTCT	840
AACCGGTCTA	TAGATCGTAA	ACGCGCAAAA	TACGAGCTTG	TTCACACCGT	GCGGGTCCAT	
ACCGGAGCAG	ATGACCGATC	CTATGAATGT	GCAACTCAAG	AAGAGGATGA		960
GTTGGGGTTT	CCTTGTCAAA	GAATCTACCA	ATGGTAGCTG	CAAGAACCCT	AAAGATCAAT	
ATCGCAACTT	TGGGTCCGCT	TGTTCTTCCC	ATAAGCGAGA	AGTTTCACTT	CTTTGTGAGG	1080
TTCGTTAAAA	AGAAGTTTCT	CAACCCCAAG	CTAAAGCATT	ACATTCCGGA	TTTCAAGCTC	
GCATTCGAGC	ATTTCTGTAT	CCATGCGGGT	GGTAGAGCGC	TAATTGATGA		1200
AATCTTCATC	TAACTCCACT	AGACGTTGAG	GCTTCAAGAA	TGACATTACA		
AATACCTCTT	CGAGCTCCAT	TTGGTACGAG	TTGGCTTACA	CAGAAGCCAA		1320
ACGAAAGGAG	ATAGGATTTG	GCAGATTGCG	TTGGGGTCAG	GTTTTAAGTG		
GTTTGGGTGG	CTCTTCGTAA	CGTCAAGCCT	TCTACTAATA	ATCCTTGGGA	ACAGTGTCTA	1440
CACAAATATC	CAGTTGAGAT	CGATATAGAT	TTAAAAGAG			

EL2 FIGURE 5

EL2 protein sequence
Molecular Weight 55799.30 Daltons
493 Amino Acids
55 Strongly Basic(+) Amino Acids (K,R)
46 Strongly Acidic(-) Amino Acids (D,E)
181 Hydrophobic Amino Acids (A,I,L,F,W,V)
134 Polar Amino Acids (N,C,Q,S,T,Y)
8.756 Isolectric Point
10.995 Charge at PH 7.0

MDYPMKKVKI FFNYLMAHRF KLCFLPLMVA IAVEASRLST QDLQNFYLYL QNNHTSLTMF FLYLALGSTL YLMTRPKPVY LVDFSCYLPP SHLKASTQRI MQHVRLVREA GAWKQESDYL MDFCEKILER SGLGQETYVP EGLQTLPLQQ NLAVSRIETE EVIIGAVDNL FRNTGISPSD IGILVVNSST FNPTPSLSSI LVNKFKLRDN IKSLNLGGMG CSAGVIAIDA AKSLLQVHRN TYALVVSTEN ITQNLYMGNN KSMLVTNCLF RIGGAAILLS NRSIDRKRAK YELVHTVRVH TGADDRSYEC ATQEEDEDGI VGVSLSKNLP MVAARTLKIN IATLGPLVLP ISEKFHFFVR FVKKKFLNPK LKHYIPDFKL AFEHFCIHAG GRALIDEMEK NLHLTPLDVE ASRMTLHRFG NTSSSSIWYE LAYTEAKGRM TKGDRIWQIA LGSGFKCNSS VWVALRNVKP STNNPWEQCL HKYPVEIDID LKE

FIGURE 6

EL3 151	2 bases					
CTACGTCAGG	GTAGAACAAA	GAGTAAACAC	TTAAGCAAAA	CAATTTGTCC	TACTCTTAGG	TTATCTCCAA
TGAAGAACTT	AAAGATGGTT	TTCTTCAAGA	TCCTCTTTAT	CTCTTTAATG	GCAGGATTAG	CCATGAAAGG
ATCTAAGATC	AACGTAGAAG	ATCTCCAAAA	GTTCTCCCTC	CACCATACAC	AGAACAACCT	CCAAACCATA .
AGCCTTCTAT	TGTTTCTTGT	CGTTTTTGTG	TGGATCCTCT	ACATGTTAAC	CCGACCTAAA	CCCGTTTACC
TTGTTGATTT	CTCCTGCTAC	CTTCCACCGT	CGCATCTCAA	GGTCAGTATC	CAAACCCTAA	TGGGACACGC
AAGACGTGCA	AGAGAAGCAG	GCATGTGTTG	GAAGAACAAA	GAGAGCGACC	ATTTAGTTGA	CTTCCAGGAG
AAGATTCTTG	AACGTTCCGG	TCTTGGTCAA	GAAACCTACA	TCCCCGAGGG	TCTTCAGTGC	TTCCCACTTC
AGCAAGGCAT	GGGTGCTTCA	CGTAAAGAGA	CGGAAGAAGT	AATCTTCGGA	GCTCTTGACA	ATCTTTTTCG
CAACACCGGT	GTAAAACCTG	ATGATATCGG	TATATTGGTG	GTGAATTCTA	GCACGTTTAA	TCCAACTCCA
TCACTCGCCT	CCATGATTGT	GAACAAGTAC	AAACTCAGAG	ACAACATCAA	GAGTTTGAAT	CTTGGAGGGA
TGGGTTGCAG	TGCCGGAGTT	ATAGCTGTTG	ATGTCGCTAA	GGGATTACTA	CAAGTTCATA	GGAACACTTA
TGCTATTGTA	GTAAGCACAG	AGAACATCAC	TCAGAACTTA	TACTTGGGGA	AAAACAAATC	AATGCTAGTC
ACAAACTGTT	TGTTCCGCGT	TGGTGGTGCT	GCGGTTCTGC	TTTCAAACAG	ATCTAGAGAC	CGTAACCGCG
CCAAATACGA	GCTTGTTCAC	ACCGTACGGA	TCCATACCGG	ATCAGATGAT	AGGTCGTTCG	AATGTGCGAC
ACAAGAAGAG	GATGAAGATG	GTATAATTGG	AGTTACCTTG	ACAAAGAATC	TACCTATGGT	GGCTGCAAGG
ACTCTTAAGA	TAAATATCGC	AACTTTGGGT	CCTCTTGTAC	TTCCATTAAA	AGAGAAGCTA	GCCTTCTTTA
TTACTTTTGT	CAAGAAGAAG	TATTTCAAGC	CAGAGTTAAG	GAATTATACA	CCAGATTTCA	AGCTTGCCTT
TGAGCATTTC	TGTATCCACG	CTGGTGGAAG	AGCTCTAATA	GATGAGCTGG	AGAAGAACCT	TAAGCTTTCT
CCGTTACACG	TAGAGGCGTC	AAGAATGACA	CTACACAGGT	TTGGTAACAC	TTCTTCTAGC	TCAATCTGGT
ACGAGTTAGC	TTATACAGAA	GCTAAAGGAA	GGATGAAGGA	AGGAGATAGG	ATTTGGCAGA	TTGCTTTGGG
GTCAGGTTTT	AAGTGTAACA	GTTCAGTATG	GGTGGCTCTG	CGAGACGTTA	AGCCTTCAGC	TAACAGTCCA
TGGGAAGACT	GTATGGATAG	ATATCCGGTT	GAGATTGATA	TT		

EL3 FIGURE 7

EL3 protein sequence
Molecular Weight 56801.10 Daltons
504 Amino Acids
66 Strongly Basic(+) Amino Acids (K,R)
48 Strongly Acidic(-) Amino Acids (D,E)
183 Hydrophobic Amino Acids (A,I,L,F,W,V)
127 Polar Amino Acids (N,C,Q,S,T,Y)
9.315 Isolectric Point
19.797 Charge at PH 7.0

LRQGRTKSKH LSKTICPTLR LSPMKNLKMV FFKILFISLM AGLAMKGSKI NVEDLQKFSL HHTQNNLQTI SLLLFLVVFV WILYMLTRPK PVYLVDFSCY LPPSHLKVSI QTLMGHARRA REAGMCWKNK ESDHLVDFQE KILERSGLGQ ETYIPEGLQC FPLQQGMGAS RKETEEVIFG ALDNLFRNTG VKPDDIGILV VNSSTFNPTP SLASMIVNKY KLRDNIKSLN LGGMGCSAGV IAVDVAKGLL QVHRNTYAIV VSTENITQNL YLGKNKSMLV TNCLFRVGGA AVLLSNRSRD RNRAKYELVH TVRIHTGSDD RSFECATQEE DEDGIIGVTL TKNLPMVAAR TLKINIATLG PLVLPLKEKL AFFITFVKKK YFKPELRNYT PDFKLAFEHF CIHAGGRALI DELEKNLKLS PLHVEASRMT LHRFGNTSS SIWYELAYTE AKGRMKEGDR IWQIALGSGF KCNSSVWVAL RDVKPSANSP WEDCMDRYPV EIDI

EL3 FIGURE 8

EL4 cDNA	1650) bases				
ATGGGTAGAT	CCAACGAGCA	AGATCTGCTC	TCTACCGAGA	TCGTTAATCG	TGGGATCGAA	CCATCCGGTC
CTAACGCCGG	CTCACCAACG	TTCTCGGTTA	GGGTCAGGAG	ACGTTTGCCT	GATTTTCTTC	AGTCGGTGAA
CTTGAAGTAC	GTGAAACTTG	GTTACCACTA	CCTCATAAAC	CATGCGGTTT	ATTTGGCGAC	CATACCGGTT
CTTGTGCTGG	TTTTTAGTGC	TGAGGTTGGG	AGTTTAAGCA	GAGAAGAGAT	TTGGAAGAAG	CTTTGGGACT
ATGATCTTGC	AACTGTTATC	GGATTCTTCG	GTGTCTTTGT	TTTAACCGCT	TGTGTCTACT	TCATGTCTCG
TCCTCGCTCT	GTTTATCTTA	TTGATTTCGC	TTGTTACAAG	CCCTCCGATG	AACACAAGGT	GACAAAAGAA
GAGTTCATAG	AACTAGCGAG	AAAATCAGGG	AAGTTCGACG	AAGAGACACT	CGGTTTCAAG	AAGAGGATCT
TACAAGCCTC	AGGCATAGGC	GACGAGACAT	ACGTCCCAAG	ATCCATCTCT	TCATCAGAAA	ACATAACAAC
GATGAAAGAA	GGTCGTGAAG	AAGCCTCTAC	AGTGATCTTT	GGAGCACTAG	ACGAACTCTT	CGAGAAGACA
CGTGTAAAAC	CTAAAGACGT	TGGTGTCCTT	GTGGTTAACT	GTAGCATTTT	CAACCCGACA	CCGTCGTTGT
CCGCAATGGT	GATAAACCAT	TACAAGATGA	GAGGGAACAT	ACTTAGTTAC	AACCTTGGAG	GGATGGGATG
TTCGGCTGGA	ATCATAGCTA	TTGATCTTGC	TCGTGACATG	CTTCAGTCTA	ACCCTAATAG	TTATGCTGTT
GTTGTGAGTA	CTGAGATGGT	TGGGTATAAT	TGGTACGTGG	GAAGTGACAA	GTCAATGGTT	ATACCTAATT
GTTTCTTTAG	GATGGGTTGT	TCTGCCGTTA	TGCTCTCTAA	CCGTCGTCGT	GACTTTCGCC	ATGCTAAGTA
CCGTCTCGAG	CACATTGTCC	GAACTCATAA	GGCTGCTGAC	GACCGTAGCT	TCAGGAGTGT	GTACCAGGAA
GAAGATGAAC	AAGGATTCAA	GGGGTTGAAG	ATAAGTAGAG	ACTTAATGGA	AGTTGGAGGT	GAAGCTCTCA
AGACAAACAT	CACTACCTTA	GGTCCTCTTG	TCCTACCTTT	CTCCGAGCAG	CTTCTCTTCT	TTGCTGCTTT
GGTCCGCCGA	ACATTCTCAC	CTGCTGCCAA	AACGTCCACA	ACCACTTCCT	TCTCTACTTC	CGCCACCGCA
AAAACCAATG	GAATCAAGTC	TTCCTCTTCC	GATCTGTCCA	AGCCATACAT	CCCGGACTAC	AAGCTCGCCT
TCGAGCATTT	TTGCTTCCAC	GCGGCAAGCA	AAGTAGTGCT	TGAAGAGCTT	CAAAAGAATC	TAGGCTTGAG
TGAAGAGAAT	ATGGAGGCTT	CTAGGATGAC	ACTTCACAGG	TTTGGAAACA	CTTCTAGCAG	TGGAATCTGG
TATGAGTTGG	CTTACATGGA	GGCCAAGGAA	AGTGTTCGTA	GAGGCGATAG	GGTTTGGCAG	ATCGCTTTCG
GTTCTGGTTT	TAAGTGTAAC	AGTGTGGTGT	GGAAGGCAAT	GAGGAAGGTG	AAGAAGCCAA	CCAGGAACAA
TCCTTGGGTG	GATTGCATCA	ACCGTTACCC	TGTGCCTCTC			

EL4 FIGURE 9

EL4 protein sequence
Molecular Weight 61953.80 Daltons
550 Amino Acids
71 Strongly Basic(+) Amino Acids (K,R)
58 Strongly Acidic(-) Amino Acids (D,E)
191 Hydrophobic Amino Acids (A,I,L,F,W,V)
147 Polar Amino Acids (N,C,Q,S,T,Y)
9.036 Isolectric Point
14.349 Charge at PH 7.0

MGRSNEODLL	STEIVNRGIE	PSGPNAGSPT	FSVRVRRRLP	DFLQSVNLKY	VKLGYHYLIN	HAVYLATIPV
LVLVFSAEVG	SLSREEIWKK	LWDYDLATVI	GFFGVFVLTA	CVYFMSRPRS	VYLIDFACYK	PSDEHKVTKE
EFIELARKSG	KFDEETLGFK	KRILQASGIG	DETYVPRSIS	SSENITTMKE	GREEASTVIF	GALDELFEKT
	VVNCSIFNPT					
VVSTEMVGYN	WYVGSDKSMV	IPNCFFRMGC	SAVMLSNRRR	DFRHAKYRLE	HIVRTHKAAD	DRSFRSVYQE
EDEQGFKGLK	ISRDLMEVGG	EALKTNITTL	GPLVLPFSEQ	LLFFAALVRR	TFSPAAKTST	TTSFSTSATA
	DLSKPYIPDY					FGNTSSSGIW
YELAYMEAKE	SVRRGDRVWQ	IAFGSGFKCN	SVVWKAMRKV	KKPTRNNPWV	DCINRYPVPL	

EL4 FIGURE 10

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EL5 cDNA	1611	bases				
TCGAGCTACG	TCAGGGCTTT	TATATGCACA	AATTCTCATA	AAGTTTTCAA	TTTTATTCCA	TTTTTCTCGG
AAGCCATGGA	AGCTGCTAAT	GAGCCTGTTA	ATGGCGGATC	CGTACAGATC	CGAACAGAGA	ACAACGAAAG
ACGAAAGCTT	CCTAATTTCT	TACAAAGCGT	CAACATGAAA	TACGTCAAGC	TAGGTTATCA	TTACCTCATT
ACTCATCTCT	TCAAGCTCTG	TTTGGTTCCA	TTAATGGCGG	TTTTAGTCAC	AGAGATCTCT	CGATTAACAA
CAGACGATCT	TTACCAGATT	TGGCTTCATC	TCCAATACAA	TCTCGTTGCT	TTCATCTTTC	TCTCTGCTTT
AGCTATCTTT	GGCTCCACCG	TTTACATCAT	GAGTCGTCCC	AGATCTGTTT	ATCTCGTTGA	TTACTCTTGT
TATCTTCCTC	CGGAGAGTCT	TCAGGTTAAG	TATCAGAAGT	TTATGGATCA	TTCTAAGTTG	ATTGAAGATT
TCAATGAGTC	ATCTTTAGAG	TTTCAGAGGA	AGATTCTTGA	ACGTTCTGGT	TTAGGAGAAG	AGACTTATCT
CCCTGAAGCT	TTACATTGTA	TCCCTCCGAG	GCCTACGATG	ATGGCGGCTC	GTGAGGAATC	TGAGCAGGTA
ATGTTTGGTG	CTCTTGATAA	GCTTTTCGAG	AATACCAAGA	TTAACCCTAG	GGATATTGGT	GTGTTGGTTG
TGAATTGTAG	CTTGTTTAAT	CCTACACCTT	CGTTGTCAGC	TATGATTGTT	AACAAGTATA	AGCTTAGAGG
GAATGTTAAG	AGTTTTAACC	TTGGTGGAAT	GGGGTGTAGT	GCTGGTGTTA	TCTCTATCGA	TTTAGCTAAA
GATATGTTGC	AAGTTCATAG	GAATACTTAT	GCTGTTGTGG	TTAGTACTGA	GAACATTACT	CAGAATTGGT
ATTTTGGGAA	TAAGAAGGCT	ATGTTGATTC	CGAATTGTTT	GTTTCGTGTT	GGTGGTTCGG	CGATTTTGTT
GTCGAACAAG	GGGAAAGATC	GTAGACGGTC	TAAGTATAAG	CTTGTTCATA	CCGTTAGGAC	TCATAAAGGA
GCTGTTGAGA	AGGCTTTCAA	CTGTGTTTAC	CAAGAGCAAG	ATGATAATGG	GAAGACCGGG	GTTTCGTTGT
CGAAAGATCT	TATGGCTATA	GCTGGGGAAG	CTCTTAAGGC	GAATATCACT	ACTTTAGGTC	CTTTGGTTCT
TCCTATAAGT	GAGCAGATTC	TGTTTTTCAT	GACTTTGGTT	ACGAAGAAAC	TGTTTAACTC	GAAGCTGAAG
CCGTATATTC	CGGATTTCAA	GCTTGCGTTT	GATCATTTCT	GTATCCATGC	TGGTGGTAGA	GCTGTGATTG
ATGAGCTTGA	GAAGAATCTG	CAGCTTTCGC	AGACTCATGT.	CGAGGCATCC	AGAATGACAC	TGCACAGATT
TGGAAACACT	TCTTCGAGCT	CGATTTGGTA	TGAACTGGCT	TACATAGAGG	CTAAAGGTAG	GATGAAGAAA
GGAAACCGGG	TTTGGCAGAT	TGCTTTTGGA	AGTGGGTTTA	AGTGTAACAG	TGCAGTTTGG	GTGGCTCTAA
ACAATGTCAA	GCCTTCGGTT	AGTAGTCCGT	GGGAACACTG	CATCGACCGA	TATCCGGTTA	AGCTCGACTT
C		,			•	

EL5 FIGURE 11

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EL5 protein sequence
Molecular Weight 60874.60 Daltons
537 Amino Acids
63 Strongly Basic(+) Amino Acids (K,R)
47 Strongly Acidic(-) Amino Acids (D,E)
198 Hydrophobic Amino Acids (A,I,L,F,W,V)
148 Polar Amino Acids (N,C,Q,S,T,Y)
9.107 Isolectric Point
17.930 Charge at PH 7.0

SSYVRAFICT	NSHKVFNFIP	FFSEAMEAAN	EPVNGGSVQI	RTENNERRKL	PNFLQSVNMK	YVKLGYHYLI
THLFKLCLVP	LMAVLVTEIS	RLTTDDLYQI	WLHLQYNLVA	FIFLSALAIF	GSTVYIMSRP	RSVYLVDYSC
YLPPESLOVK	YOKFMDHSKL	IEDFNESSLE	FORKILERSG	LGEETYLPEA	LHCIPPRPTM	MAAREESEQV
MFGALDKLFE	NTKINPRDIG	VLVVNCSLFN	PTPSLSAMIV	NKYKLRGNVK	SFNLGGMGCS	AGVISIDLAK
DMLOVHRNTY	AVVVSTENIT	QNWYFGNKKA	MLIPNCLFRV	GGSAILLSNK	GKDRRRSKYK	LVHTVRTHKG
AVEKAFNCVY	QEQDDNGKTG	VSLSKDLMAI	AGEALKANIT	TLGPLVLPIS	EQILFFMTLV	TKKLFNSKLK
PYIPDFKLAF	DHFCIHAGGR	AVIDELEKNL	QLSQTHVEAS	RMTLHRFGNT	SSSSIWYELA	YIEAKGRMKK
GNRVWOIAFG	SGFKCNSAVW	VALNNVKPSV	SSPWEHCIDR	YPVKLDF		

EL5 FIGURE 12

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1502 bases EL6 TCTCCGACGATGCCTCAGGCACCGATGCCAGAGTTCTCTAGCTCGGTGAAGCTCAAGTACGTGAAACTTGGTTACCAA TATTTGGTTAACCATTTCTTGAGTTTTCTTTTGATCCCGATCATGGCTATTGTCGCCGTTGAGCTTCTTCGGATGGGT TTCATCTCCACTGTTTACTTCATGTCCAAGCCACGCACCATCTACCTCGTTGACTATTCTTGTTACAAGCCACCTGTC ACGTGTCGTGTCCCCTTCGCAACTTTCATGGAACACTCTCGTTTGATCCTCAAGGACAAGCCTAAGAGCGTCGAGTTC $\verb|CCAACCATGGACGCGGCTAGAAGCGAGGCTCAGATGGTTATCTTCGAGGCCATGGACGATCTTTTCAAGAAAACCGGT|\\$ CTTAAACCTAAAGACGTCGACATCCTTATCGTCAACTGCTCTCTTTTCTCTCCCACACCATCGCTCTCAGCTATGGTC ATCAACAAATATAAGCTTAGGAGTAATATCAAGAGCTTCAATCTTTCGGGGATGGGCTGCAGCGCGGGCCTGATCTCA GTTGATCTAGCCGCGACTTGCTCCAAGTTCATCCCAATTCAAATGCAATCATCGTCAGCACGGAGATCATAACGCCT AATTACTATCAAGGCAACGAGAGCCATGTTGTTACCCAATTGTCTCTCCGCATGGGTGCGGCAGCCATACACATG TCAAACCGCCGGTCTGACCGGTGGCGAGCCAAATACAAGCTTTCCCACCTCGTCCGGACACACCGTGGCGCTGACGAC ATCGCCGGTGAAGCCCTCAAGGCAAACATCACCACAATAGGTCCTTTGGTCCTACCGGCGTCAGAACAACTTCTCTTC CACTTTTGCATTCACGCAGGAGCCAGAGCGGTGATCGACGAGCTCCAAAAGAATCTACAACTATCAGGAGAACACGTT GAGGCCTCAAGAATGACACTACATCGTTTTGGTAACACGTCATCTTCATCGTTATGGTACGAGCTTAGCTACATCGAG TCTAAAGGGAGAATGAGGAGAGGCGATCGCGTTTGGCAAATCGCGTTTGGGAGTGGTTTCAAGTGTAACTCTGCCGTG CCCGAAGTTGTCAAACTCTA

> EL6 FIGURE 13

EL6 protein sequence
Molecular Weight 56687.90 Daltons
500 Amino Acids
59 Strongly Basic(+) Amino Acids (K,R)
46 Strongly Acidic(-) Amino Acids (D,E)
182 Hydrophobic Amino Acids (A,I,L,F,W,V)
127 Polar Amino Acids (N,C,Q,S,T,Y)
8.909 Isolectric Point
14.567 Charge at PH 7.0

SPTMPQAPMP EFSSVKLKY VKLGYQYLVN HFLSFLLIPI MAIVAVELLR MGPEEILNVW NSLQFDLVQV LCSSFFVIFI STVYFMSKPR TIYLVDYSCY KPPVTCRVPF ATFMEHSRLI LKDKPKSVEF QMRILERSGL GEETCLPPAI HYIPPTPTMD AARSEAQMVI FEAMDDLFKK TGLKPKDVDI LIVNCSLFSP TPSLSAMVIN KYKLRSNIKS FNLSGMGCSA GLISVDLARD LLQVHPNSNA IIVSTEIITP NYYQGNERAM LLPNCLFRMG AAAIHMSNRR SDRWRAKYKL SHLVRTHRGA DDKSFYCVYE QEDKEGHVGI NLSKDLMAIA GEALKANITT IGPLVLPASE QLLFLTSLIG RKIFNPKWKP YIPDFKLAFE HFCIHAGGRA VIDELQKNLQ LSGEHVEASR MTLHRFGNTS SSSLWYELSY IESKGRMRRG DRVWQIAFGS GFKCNSAVWK CNRTIKTPKD GPWSDCIDRY PVFIPEVVKL

EL6 FIGURE 14

1548 bases ${\tt ATGGACGGTGCCGGAGAATCACGACTCGGTGGTGGTGGTGGTGGTGGTGGTTCTGTTGGAGTTCAGATCCGACAAACA}$ CGGATGCTACCGGATTTTCTCCAGAGCGTGAATCTCAAGTATGTGAAATTAGGTTACCATTACTTAATCTCAAATCTC TTGACTCTCTGTTTATTCCCTCTCGCCGTTGTTATCTCCGTCGAAGCCTCTCAGATGAACCCAGATGATCTCAAACAG $\tt CTCTGGATCCATCTACAATACAATCTGGTTAGTATCATCATCTGTTCAGCGATTCTAGTCTTCGGGTTAACGGTTTAT$ GTTATGACCCGACCTAGACCCGTTTACTTGGTTGATTTCTCTTGTTATCTCCCACCTGATCATCTCAAAGCLCCTTAC GCTCGGTTCATGGAACATTCTAGACTCACCGGAGATTTCGATGACTCTCGCTCTCGAGTTTCAACGCAAGATCCTTGAG CGTTCTGGTTTAGGGGAAGACACTTATGTCCCTGAAGCTATGCATTATGTTCCACCGAGAATTTCAATGGCTGCTGCT ${\tt AGAGAAGAAGCTGAACAAGTCATGTTTGGTGCTTTAGATAACCTTTTCGCTAACACTAATGTGAAACCAAAGGATATT}$ ${\tt GGAATCCTTGTTGTGAATTGTAGTCTCTTTAATCCAACTCCTTCGTTATCTGCAATGATTGTGAACAAGTATAAGCTT}$ AGAGGTAACATTAGAAGCTACAATCTAGGCGGTATGGGTTGCAGCGCGGGAGTTATCGCTGTGGATCTTGCTAAAGAC ATGTTGTTGGTACATAGGAACACTTATGCGGTTGTTGTTTCTACTGAGAACATTACTCAGAATTGGTATTTTGGTAAC AAGAAATCGATGTTGATACCGAACTGCTTGTTTCGAGTTGGTGGCTCTGCGGTTTTGCTATCGAACAAGTCGAGGGAC AAGAGACGGTCTAAGTACAGGCTTGTACATGTAGTCAGGACTCACCGTGGAGCAGATGATAAAGCTTTCCGTTGTGTT TATCAAGAGCAGGATGATACAGGGAGAACCGGGGTTTCGTTGTCGAAAGATCTAATGGCGATTGCAGGGGAAACTCTC AAAACCAATATCACTACATTGGGTCCTCTTGTTCTACCGATAAGTGAGCAGATTCTCTTCTTTATGACTCTAGTTGTG ${\tt AAGAAGCTCTTTAACGGTAAAGTGAAACCGTATATCCCGGATTTCAAACTTGCTTTCGAGCATTTCTGTATCCATGCT}$ ${\tt GGTGGAAGAGCTGTGATCGATGAGTTAGAGAAGAATCTGCAGCTTTCACCAGTTCATGTCGAGGCTTCGAGGATGACT}$ $\tt CTTCATCGATTTGGTAACACATCTTCGAGCTCCATTTGGTATGAATTGGCTTACATTGAAGCGAAGGGAAGGATGCGA$ GTGAAACCTTCGAACAACAGTCCTTGGGAAGATTGTATTGACAAGTATCCGGTAACTTTAAGTTAT

> EL7 FIGURE 15

EL7 protein sequence
Molecular Weight 57848.80 Daltons
516 Amino Acids
59 Strongly Basic(+) Amino Acids (K,R)
48 Strongly Acidic(-) Amino Acids (D,E)
189 Hydrophobic Amino Acids (A,I,L,F,W,V)
131 Polar Amino Acids (N,C,Q,S,T,Y)
8.872 Isolectric Point
12.792 Charge at PH 7.0

MDGAGESRLG GDGGGDGSVG VQIRQTRMLP DFLQSVNLKY VKLGYHYLIS NLLTLCLFPL AVVISVEASQ MNPDDLKQLW IHLQYNLVSI IICSAILVFG LTVYVMTRPR PVYLVDFSCY LPPDHLKAPY ARFMEHSRLT GDFDDSALEF QRKILERSGL GEDTYVPEAM HYVPPRISMA AAREEAEQVM FGALDNLFAN TNVKPKDIGI LVVNCSLFNP TPSLSAMIVN KYKLRGNIRS YNLGGMGCSA GVIAVDLAKD MLLVHRNTYA VVVSTENITQ NWYFGNKKSM LIPNCLFRVG GSAVLLSNKS RDKRRSKYRL VHVVRTHRGA DDKAFRCVYQ EQDDTGRTGV SLSKDLMAIA GETLKTNITT LGPLVLPISE QILFFMTLVV KKLFNGKVKP YIPDFKLAFE HFCIHAGGRA VIDELEKNLQ LSPVHVEASR MTLHRFGNTS SSSIWYELAY IEAKGRMRG NRVWQIAFGS GFKCNSAIWE ALRHVKPSNN SPWEDCIDKY PVTLSY

EL7 FIGURE 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11384

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IPC(6)	IPC(6) :AO1H 5/00, C07H 21/00; C12N 15/00, 15/82						
	:800/205; 435/172.3; 536/23.6 o International Patent Classification (IPC) or to both	national classification and IPC					
	DS SEARCHED						
<u> </u>	ocumentation searched (classification system follows	ed by classification symbols)					
	800/205; 435/172.3; 536/23.6	, ,	-				
Documentat	Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched						
Electronic d	ata base consulted during the international search (rALOG	ame of data base and, where practicable,	search terms used)				
C. DOC	C. DOCUMENTS CONSIDERED TO BE RELEVANT						
Category*	Category* Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No.						
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X Furth	er documents are listed in the continuation of Box (C. See patent family annex.					
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